REDUCTION OF POPULATIONS OF *PHYTOPHTHORA* SPP. WITH SOIL SOLARIZATION UNDER FIELD CONDITIONS AND THERMAL INACTIVATION OF *PHYTOPHTHORA NICOTIANAE*

By

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To Eliamar C. M. Coelho,

who has encouraged and supported me through the years.

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Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
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REDUCTION OF POPULATIONS OF *PHYTOPHTHORA* SPP. WITH SOIL SOLARIZATION UNDER FIELD CONDITIONS AND THERMAL INACTIVATION OF *PHYTOPHTHORA NICOTIANAE*

By

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The effects of soil solarization in combination with cabbage amendments on the survival of populations of *Phytophthora* spp. were evaluated in North Florida. Soil under solarization for 45 to 55 days reached a maximum temperature of 47°C on up to 10 days at 10 cm of depth. Soil solarization with a clear, gas impermeable, low density polyethylene film was as effective as methyl bromide in reducing populations of *P. nicotianae* at a depth of 10 cm; however, at 25 cm of depth the population of the pathogen was similar to that in the control treatments. Reduction of survival of *P. capsici* in two sites in 1994 under solarization with either a clear, low density polyethylene or a clear, gas impermeable, low density polyethylene film was as effective as the methyl bromide treatment at the 10-cm depth, while at the 25-cm depth no control was observed. Incorporation of cabbage into the soil at a rate of 70 to 90 metric tons per hectare did not enhance the effectiveness of solarization.

Studies on the thermal inactivation of *P. nicotianae* in the laboratory confirmed that the time required to inactivate chlamydospores of the pathogen is inversely proportional to the temperature of the treatment. The time required to reduce soil populations from 500 propagules per gram of soil to residual levels (0.2 propagule per gram of soil or less) was 10 minutes, 45 minutes, 4 hours, 12 hours, 4 days, and 16 days at 53°, 50°, 47°, 44°, 41° and 38°C, respectively. The incorporation of cabbage into the soil reduced the time required to inactive the chlamydospores of *P. nicotianae*. Detection of *P. nicotianae* by the tomato-seedling baiting technique provided similar results to the soil plating procedure, except when only residual populations were present in the soil.

Temperature regimes that simulated solarization periods were effective in eliminating *P. nicotianae* only when optimum regimes were used (47°C for 3 hours daily and 44°C for 5 hours daily). Populations of *P. nicotianae* decreased at matric potentials of 0, -10, and -30 kPa with time of exposure to each temperature regime, but the lowest survival occurred in saturated soil.

Soils with different cropping history and pasteurization treatment may have marked differences on the survival of *P. nicotianae*. Nonpasteurized soils from a fallow field, coupled with optimum temperature regimes, provided the greatest reduction in survival of the pathogen in relation to two other soils from commercial cropping systems.

CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW

Control of plant diseases caused by soilborne pathogens constitutes one of the most difficult aspects of disease management. In general, plant disease control is based on six principles: avoidance, exclusion, eradication, protection, disease resistance, and therapy (Maloy, 1993). Once a pathogen has been introduced in an area, efforts are generally directed to eradicating it or protecting the plant from disease. Tactics for control of diseases include disease resistance, crop rotation, and other cultural practices, or the use of chemicals to protect plants. For high value crops, one of the most effective methods for the control of soilborne plant pathogens is fumigation of soil with methyl bromide or other chemicals. The imminent ban on the production and use of methyl bromide has prompted a search for other alternatives for disease control. Among these strategies, removing a field from production for a considerable period would allow the reduction of populations of pathogens by attrition. However, this strategy may not be effective for pathogens that form resting structures that can survive for long periods in the absence of the host plant (Baker and Cook, 1974). The use of plastic films for solarization of soil in agriculture has opened new horizons for the control of soilborne plant pathogens. Solarization raises the soil temperature to levels that are lethal to most mesophyllic organisms; thus, soilborne plant pathogens of many crops may be controlled. The mechanisms by which disease control is achieved with this method are not fully understood and warrant further studies.

Soil solarization is a hydrothermal process in which moist soil is covered with transparent plastic and exposed to sunlight, allowing it to heat to temperatures under

favorable conditions that are lethal to many plant pathogens, pests and weeds (DeVay, 1991b). The effectiveness of soil solarization depends on soil color and structure, soil moisture; air temperature, length of day, and intensity of sunlight (DeVay, 1991b). The process has been studied during the last 20 years as an alternative to chemical fumigation of soil for the management of soilborne plant pathogens (Katan, 1980, 1981; Katan *et al.*, 1987). Solarization may reduce populations of several fungi, such as *Fusarium* spp. (Chellemi *et al*, 1994; Katan *et al.*, 1976, Katan *et al.*, 1983; Porter and Merriman, 1985; Ramirez-Villapudua and Munnecke, 1987, 1988), *Phytophthora* spp. (Barbercheck and Von Broembsen, 1986; Chellemi *et al*, 1994; Hartz *et al.*, 1993; Juarez-Palacios *et al.*, 1991; Kassaby, 1985; McGovern and Begeman, 1996; Moens and Aicha, 1990; Pinkas *et al.*, 1984; Wicks, 1988), *Pythium ultimum* (Gamliel and Stapleton, 1993a, 1993b; Gamliel *et al.*, 1989, 1993; Kulkarni *et al.*, 1992; Pullman *et al.*, 1981a, 1981b; Stapleton and Garza-Lopez, 1988; Stapleton *et al.*, 1995), *Verticillium dahliae* (Ghini *et al.*, 1993; Hartz *et al.*, 1993; Katan *et al.*, 1976; Porter and Merriman, 1985; Pullman *et al.*, 1981a, 1981b), and *Rhizoctonia* sp. (Gamliel *et al.*, 1993; Grooshevoy *et al.*, 1941; Keinath, 1995; Lewis and Papavizas, 1974; Pullman *et al.*, 1981a, 1981b). However, disease management is not always achieved, as exemplified by the study of Stapleton and Garza-Lopez (1988) in which a reduction in the population of *Macrophomina phaseolina* did not result in less disease in the indicator crop. Other soilborne pathogens that have not been controlled by soil solarization include *Plasmodiophora brassicae, Sclerotium rolfsii*, *Pythium aphanidermatum* and many others, including *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Chellemi *et al.*, 1994; Stapleton and DeVay, 1986).

Success of solarization for the control of plant diseases is closely associated with a combination of high ambient temperatures, maximum solar radiation, and optimum soil moisture (DeVay, 1991b; Souza, 1994). Early demonstration of the use of solarization came from work in arid climates by Katan (1980, 1981) and Katan *et al.* (1976, 1987) in Israel, by Stapleton and DeVay (1984, 1986) in California, and in semi-arid climates in

Mexico by Stapleton and Garza-Lopez (1988) . Regions where the summer coincides with the rainy season, such as in the southeastern U.S.A., may have less potential for successful solarization because of the cooling effect of frequent rain showers, as well as extended cloud cover, which reduce the solar radiation captured under the plastic tarp (Chellemi *et al.*, 1994). Soil solarization has been used successfully in the Southeastern U.S.A. to manage *Rhizoctonia solani*, *Didymella bryoniae*, *Fusarium* spp. and *P. nicotianae* (Chellemi *et al.*, 1994; Keinath, 1995, 1996). Populations of *P. nicotianae* were reduced to undetectable levels in soil depths of up to 15 cm in three sites in North Florida. However, reduction in populations below 25 cm of depth was noted in only one site. In contrast, methyl bromide nearly completely eradicated the pathogen in depths of up to 35 cm. Control of *Fusarium* spp. was limited to the top 5 cm of the soil, and no control was achieved at some sites (Chellemi *et al.*, 1994). Keinath (1995) found that the number of organic fragments colonized by *R. solani* was lower in solarized than in nonsolarized soils; however, the control of belly rot on pickling cucumber was not as effective with solarization as was the chemical treatment with chlorothalonil.

The type of plastic used for solarization may also influence soil solarization (DeVay, 1991a, 1991b; Malathrakis and Loulakis, 1989; Staplepton and DeVay, 1986). Stapleton and DeVay (1986) reported that thinner polyethylene sheets (25 μm) were more effective than thicker sheets (50-100 μm) for the transmission of solar radiation to the soil. DeVay (1991) noted that black polyethylene films lasted longer than transparent films that had not received a treatment for protection against UV rays; however, soil temperatures did not raise as much under the black films. Malathrakis and Loulakis (1989) compared the effectiveness of a clear, low density polyethylene film to a film of co-extruded polyethylene plus ethylene vinyl acetate (thermoplast). These authors found that the thermoplast was more effective than the clear film in raising soil temperatures and in trapping gases used for fumigation. Similarly, Chellemi *et al.* (1997) found that a gas impermeable film, consisting of a polyamide core sandwiched between two layers of

polyethylene, was more effective than a co-extruded, white-on-black film for the reduction of populations of *Paratrichodorus minor* and *Criconemella* spp. and control of Fusarium wilt on tomatoes.

Indirect effects of soil solarization may include reduced fungistasis, or induction of shifts of microbial populations that affect the survival of spores of plant pathogens. Katan *et al.* (1976) studied the effect of heating soils to 45° to 50°C on the fungistasis of *Fusarium oxysporum* f. sp. *lycopersici*. These authors observed that autoclaving or preheating soil to 50°C for 3 hours and cooling it to 25°C before infestation induced an immediate increase in germination of conidia of *F. oxysporum* f. sp. *lycopersici* incubated during the first 24 hours. However, when conidia were incubated for longer periods of time (3 to 9 days) in pasteurized soils, the final population was reduced by 81%. The following three mechanisms were suggested to explain the increased control of pathogens in solarized or pasteurized soils: fungistasis may be partially nullified at 45° to 50°C, allowing spores to germinate and then starve in the absence of the host or be killed by the existing microbiota; sublethal temperatures may weaken the resting structures, rendering them more vulnerable to antagonistic microbiota; a microbial population shift is induced which favors thermophylic saprophytes over pathogens.

Soil solarization alone may not be effective or consistent for the control of soilborne pathogens, especially in regions where the rainy season occurs simultaneously with the warmer months of the year. In such cases, the use of soil amendments may enhance the performance of solarization (Gamliel and Stapleton, 1993a; Keinath, 1996; Ramirez-Villapudua and Munnecke, 1988). Cruciferous residues, due to their high content of isothiocyanates and aldehydes, have been suggested as amendments for use in combination with solarization (Mayton *et al.*, 1996); cabbage is the primary amendment that has been studied in combination with soil solarization.

Lewis and Papavizas (1971) studied the effect of vapors from cabbage decomposition on the control of *Aphanomyces euteiches*, and found that root rot of peas

could be controlled with either cabbage amendment or with sulfur-containing volatile compounds. A later study on the survival of *R. solani* by these authors (Lewis and Papavizas, 1974) indicated that the major effect of the organic amendment resulted from either a change of the soil pH or from the low C:N ratio of the amendment, since fresh corn amendment produced similar results as the cabbage amendment.

Gamliel and Stapleton (1993b) characterized the antifungal volatiles from cabbage residue during solarization. The kinds of volatiles released from heated or nonheated soils differed, and the concentration of volatiles peaked during the first 2 weeks of heating. Spores of *P. ultimum* and sclerotia of *S. rolfsii* failed to germinate after exposure to cabbage volatiles for 20 days. The use of a crucifer amendment associated with sublethal heating (38°C) reduced germination of *P. ultimum* and *S. rolfsii in vitro* (Stapleton *et al.*, 1995). It is possible that the major benefits of the soil amendment with crucifer residues are associated with high but not lethal temperatures, when the heat alone is not sufficient to inactivate the pathogen; however, this aspect of the use of cabbage amendment has not been explored fully.

Solarization associated with cabbage amendments was evaluated by Keinath (1996) and Ramirez-Villapudua and Munnecke (1987, 1988). The volatiles released by cabbage alone reduced the populations of *F. oxysporum* f. sp. *conglutinans*; however, the association of solarization with the amendment was the most effective treatment for the control of this pathogen (Ramirez-Villapudua and Munnecke, 1987, 1988). High concentrations of amendment had no effect on the subsequent cabbage crop, but phytotoxicity was observed when tomato seedlings were transplanted into the treated soil.

Beneficial changes in soil microbiota have been suggested as one of the attributes of soil solarization, regardless of amendments; these changes may lead to higher yields or to longer periods in which the crop is not affected by the pathogen (Katan, 1981; Stapleton, 1991; Stapleton and DeVay, 1986). Solarization of soil amended with cabbage induced an increase in the populations of thermotolerant fungi and *Bacillus* spp., a

corresponding decrease in populations of fluorescent *Pseudomonas* spp. and *Fusarium* spp., and did not affect the populations of actinomycetes. These changes in populations were correlated to a higher yield of watermelon (Keinath, 1996).

Although soil solarization is a hydrothermal process, depending on moisture for maximum heat transfer throughout the soil profile and to soilborne organisms (DeVay, 1991a; Mahrer *et al.*, 1984), very little is known about the influence of moisture on the inactivation of soilborne pathogens. Moisture has been provided during soil solarization by different methods. Kulkarni *et al.* (1992) flood-irrigated solarized plots once a week, removing the plastic tarp. These authors found that recontamination of the solarized plots could occur with either contaminated water or soil movement with the irrigation water. Furrow irrigation of level fields has been suggested as an alternative to periodically replenish moisture to the soil under solarization (Pullman *et al.*, 1979; Stapleton and DeVay, 1986). However, a single irrigation before the plastic is laid down can provide the same control of soilborne plant pathogens as several irrigation events (Grinstein *et al.*, 1979a, 1979b; Jacobsen *et al.*, 1980; Katan *et al.*, 1980; Pullman *et al.*, 1979).

Studies of thermal inactivation of pathogens can yield important information on survival of specific types of propagules and, more importantly when simulating field conditions, can serve as the basis for estimation of the time needed for soil solarization to be an effective strategy for management of soilborne plant pathogens. Relationships between the effectiveness of soil solarization in the field and thermal inactivation *in vitro* have been studied with several pathogens (Benson, 1978; Myers *et al.*, 1983; Pullman *et al.*, 1981a). However, there was a lack of quantification of the inoculum added or recovered after the heat treatment. Additionally, the propagules used in some studies for heat treatment were not the same as the propagules normally found in the soil, or the detection techniques did not allow the quantification of the surviving population (Ramirez-Villapudua and Munnecke, 1987,1988). Katan (1985) indicated the need for caution in the interpretation of the significance of heat mortality curves obtained under

laboratory conditions. Potential problems that need to be addressed include inoculum type, moisture level, medium containing the inoculum, and the procedure for heating the samples.

Early studies of thermal inactivation were more concerned with food processing, and very high temperatures were employed for short periods of time (Bigelow, 1921; Bigelow and Esty, 1920). While the emphasis at the time was on higher temperatures, Smith (1923) demonstrated that spores of *Botrytis cinerea* in aqueous solution could be inactivated at temperatures as low as 37°C whenever exposure was long enough; spores, as an example, were inactivated over a range varying from 6 minutes at 50.3°C to 23 hours at 37°C.

A logarithmic relationship between time and temperature in the inactivation of propagules has been observed (Bigelow, 1921; Bigelow and Esty, 1920; Pullman *et al.*, 1981a; Smith, 1923). Pullman *et al.* (1981a) found that the exposure time in which 90% of the oospores of *Pythium ultimum* were killed (LD_{90}) was, for each set of temperatures, 30 minutes at 50°C, 110 minutes at 47°C, 8 hours at 45°C, 42 hours at 42°C, 13 days at 38.5°C and 26 days at 37°C.

Juarez-Palacios *et al.* (1991) found that the determination of the heat sensitivity of isolates of *Phytophthora* spp. in the laboratory closely reflected their inactivation in solarized soil, and the results supported the possible use of soil solarization for management of these pathogens. These authors found that an isolate of *P. megasperma* tolerant to high temperatures survived exposure for 30 minutes at 45°C in a heat sensitivity study, but that the number of leaf discs infected by the pathogen after solarization for 4 weeks declined. In contrast *P. cinnamomi* and a low-temperature isolate of *P. megasperma* did not survive either treatment.

Bollen (1985) noted that different types of propagules have different sensitivities to heat. Oospores of *P. capsici* were more thermotolerant than mycelium from either mating type of this pathogen. The difference in tolerance was more than 5°C; oospores

survived at 50°C for 30 minutes, but mycelium was eliminated at 42.5° to 45°C for 30 minutes. Differences in the survival of *F. oxysporum* were also observed; survival of this pathogen in soil cultures was greater at higher temperatures than survival of the pathogen in soil infested before the heat treatment.The difference may have been due to the presence of chlamydospores in the soil culture. However, the system used did not reflect field conditions, since soil was continuously flooded, which rarely occurs for extended duration in any field. Bollen (1985) did not analyze the relationship of time to temperature, and the temperature used, 50°C, is reached most commonly under soil solarization only in arid or tropical climates.

One of the first attempts to control plant pathogens by exposure to intermittent heat was done by Grooshevoy *et al.* (1941). These authors demonstrated that exposing chlamydospores of *Thielaviopsis basicola* or sclerotia of a *Rhizoctonia* sp. or *Sclerotinia sclerotiorum* for 3 hours a day at 45°C for 5 days was sufficient to prevent the germination of the spores. However, no attempts were made to simulate the diurnal temperature fluctuations. Porter (1991) evaluated the effect of intermittent heat on the control of sclerotia of *S. sclerotiorum* in soil, and found that continuous heat was more effective than intermittent heat at reducing the number of viable sclerotia when the infested soil was heated for 6 hours daily over 14 days to temperatures ranging from 30° to 45°C. In a similar study with several pathogens, Porter and Merriman (1983) observed that sclerotia of *S. rolfsii* tolerated exposure to 50°C for 6 hours a day for 2 weeks, while *Sclerotium cepivorum* and *Sclerotinia minor* survived only under similar exposures at temperatures of 45°C or less. *Pythium irregulare* and *F. oxysporum* survived at 50°C, but *Verticillium dahliae* was killed at temperatures above 40°C; however, the type of fungal propagules used for this study was not determined (Porter and Merriman, 1983). Due to the labor-intensive nature of these studies and the demand for equipment, very little research has been done on the quantitative relationship of intermittent heat to the inactivation of spores of plant pathogens.

Phytophthora nicotianae Breda de Haan (syn. = *P. parasitica* Dastur) has been reported on more than 170 plant hosts in Florida (Alfieri *et al.*, 1994). Soilborne diseases caused by *P. nicotianae* have limited production of several important crops, such as citrus, tobacco, ornamentals, and tomato.

Low levels of inoculum of *P. nicotianae* in the field can result in severe epidemics (Ferrin and Mitchell, 1986a; Kannwischer and Mitchell, 1981; Mitchell, 1978). Kannwischer and Mitchell (1981) found that 0.13 chlamydospore of *P. nicotianae* per gram of soil or 42 zoospores per plant were sufficient to cause 50% mortality on a susceptible cultivar of tobacco in a controlled environment. Residual population densities of 0.005 to 0.67 propagules per gram of soil were found in a tobacco nursery; mortality of a susceptible cultivar transplanted to this field reached 80% at the end of the growth season (Ferrin and Mitchell, 1986a).

Moisture plays an important role in the formation of sporangia, zoospore release, and subsequent epidemics. Sporangia of *P. nicotianae* are produced over a range of soil matric potentials (-4 to -1500 kPa), with the greatest number of sporangia being produced at the higher end of the range (-4 to -25 kPa) (Bernhardt and Grogan, 1982; Sidebottom and Shew, 1985a). Although flooding does not favor the formation of sporangia, it enhances zoospore release (Bernhardt and Grogan, 1982).

Lutz and Menge (1991) observed that populations *of P. nicotianae* increased from 17 propagules per gram of soil before irrigation to 70 propagules per gram 2 days after a 24-hour furrow irrigation event in a citrus grove. The highest proportion of propagules at that time was comprised of sporangia and zoospores; chlamydospores increased 4 days after the irrigation event, and reached a maximum on the seventh day. When the citrus grove was irrigated with a drip system, the soil matric potential was maintained close to -10 kPa, and the highest proportion of propagules consisted of sporangia and zoospores, with populations ranging from 71 to 93 propagules per gram of soil.

Ristaino *et al* (1988) analyzed the effect of irrigation frequency and duration on the development of Phytophthora root rot of tomato and found that an irrigation regime in which plants were irrigated every 14 days, either for 4 to 8 hours or for 24 hours, increased root infection and decreased tomato yield. A less frequent irrigation schedule, such as 4 to 8 hours every 28 days, stressed the plants and also led to severe root infection. Zoospores of *P. nicotianae* can be carried in irrigation water and infect tomato plants more than 60 meters from inoculum sources (Neher and Duniway, 1992). In tomato, earlier infection led to higher disease intensity, and lower yield (Neher and Duniway, 1991; Neher *et al*., 1993). Infection of tobacco was favored by flooding of potted plants, or by saturating soil in Buchner funnel tension plates (Shew, 1983; Sidebottom and Shew, 1985b), and coincided with periods of high moisture levels in field trials (Ferrin and Mitchell, 1986b), indicating that zoospore movement is enhanced in saturated soils.

Temperature is another important factor in the development of epidemics caused by *P. nicotianae*. Lutz *et al*. (1991) found that heating naturally infested soils from citrus groves above 12°C increased germination of chlamydospores. Stimulation was observed up to 34°C, with maximum germination at 24°C and minimum at 36°C. However, such a stimulus could be due to a temperature differential between the temperature in which the spores were formed and a temperature that would provide maximum germination, and not only an increase in temperature.

Even though extensive studies have been done on the relationships of inoculum density and disease, water status of the soil and disease, and temperature and increased recovery of chlamydospores in the soil (Ferrin and Mitchell, 1986b; Lutz and Menge, 1991; Ristaino *et al*., 1988; Shew, 1983; Sidebottom and Shew, 1985a, 1985b), there is a lack of information about the effects of interactions of moisture and temperature on the inactivation of *P. nicotianae*.

Phytophthora capsici Leonian was first described infecting chile pepper in New Mexico (Leonian, 1922). This pathogen has a broad host range, including perennial plants, such as cacao, rubber, and macadamia, and annuals, such as peppers, cucurbits and several solanaceous hosts (Alfieri *et al.*, 1994). Important diseases caused by *P. capsici* in the United States include root rot, blight and fruit rot of pepper, eggplant, cantaloupe, squash and watermelon (McGovern *et al.*, 1993). In 1994, severe outbreaks of *P. capsici* were reported on tomato in South Florida (Simone, personal information).

Phytophthora capsici survives in low numbers in soil (Papavizas *et al.*, 1981). The effects of soil temperature and soil-water matric potential on survival of oospores, sporangia, and zoospores, as well as on oospores or mycelium in plant tissue, were studied by Bowers *et al.* (1990a). These authors found that zoospores survived no more than 3 weeks in field soil during the summer, when temperatures ranged from 20° to 30°C; sporangia survived up to 8 weeks. Very little decrease in viability of the oospores was observed during the same period. In a winter test, viability of oospores free of organic residue in soil decreased from 67% to less than 10% after 27 weeks, while oospores in plant tissue did not survive more than 8 weeks.

Although long-term survival of *P. capsici* in the soil occurs in the form of oospores (Bowers *et al.*, 1990a), the primary infective structures generally are zoospores (Bowers and Mitchell, 1990; Hord and Ristaino, 1992; Luz and Mitchell, 1994; Ristaino *et al.*, 1992). These authors found that flooding soils infested with oospores for as little as 2 hours resulted in increased disease, and several flooding events led to the eventual infection of all plants. Bernhardt and Grogan (1982) demonstrated that sporangia are formed within 24 hours in soils held at -30 kPa, and sporangia released zoospores 4 hours after the soil was flooded. Schlub (1983) observed that *P. capsici* spread in the field with rain splash. Bowers *et al.* (1990b) and Ristaino (1991) confirmed the influence of rainfall on disease, but noted that the movement of water over the soil surface or the plastic mulch had the most significant impact on disease development.

Knowledge of the primary survival and infection structures may be very important in the determination of strategies for the control of *P. capsici*. Still, the effects of moisture and temperature on the survival of this pathogen in the absence of a host plant are not known.

The objectives of this study were to determine the efficacy of soil solarization and organic amendment with cabbage for the control of *P. nicotianae* and *P. capsici* in the field; and to determine the relationships of time of exposure to temperature, cabbage amendment, soil water matric potential, and different soils on the thermal inactivation of propagules of *P. nicotianae* and its pathogenicity in tomato.

CHAPTER 2
THE EFFECT OF SOIL SOLARIZATION ON POPULATIONS OF *PHYTOPHTHORA NICOTIANAE* AND *P. CAPSICI* UNDER FIELD CONDITIONS

Introduction

Phytophthora nicotianae Breda de Haan (syn.= *P. parasitica* Dastur) has been reported on more than 170 plant hosts in Florida (Alfieri *et al.*, 1994). Soilborne diseases caused by *P. nicotianae* have limited production of several important crops, such as citrus, tobacco, ornamentals and tomato (Erwin and Ribeiro, 1996).

Phytophthora capsici Leonian is an important pathogen on several solanaceous plants. Severe losses have resulted from root rot, blight, and fruit rot of pepper, eggplant, cantaloupe, squash, and watermelon (McGovern *et al.*, 1993). Outbreaks of *P. capsici* have been observed in tomato, especially when soils are saturated with moisture.

Because these pathogens can survive in the soil for long periods of time in the absence of their hosts, or when weather conditions are not favorable for disease (Erwin and Ribeiro, 1996), the main strategy used by growers to reduce losses due to these pathogens, especially at the early stages of plant development, is the use of preplant fumigation with methyl bromide and application of fungicides.

The implication of methyl bromide as an ozone depleting substance has prompted a search for new alternatives for the control of soilborne plant pathogens. Soil solarization has been used in areas with arid climates for the management of soilborne diseases of high value crops (Hartz *et al.*, 1993; Pullman *et al.*, 1981b). Solarization has been evaluated in areas where the climate is considered marginally suitable for the procedure but results were not completely satisfactory (McSorley and Parrado, 1986). Soil amendments have been tested as components of soil solarization to enhance its

effectiveness (Chellemi *et al.*, 1997; Gamliel and Stapleton, 1993a, 1993b). Cabbage residues have been shown to be a potential amendment for the control of soilborne plant pathogens due to the isothiocyanates and other volatiles that are released during the heating process (Gamliel and Stapleton, 1993a; Keinath, 1996; Ramirez-Villapudua, 1987, 1988; Stapleton *et al.*, 1995) and could be useful in areas where the soil temperatures are not high enough to pasteurize the soil.

The objective of this study was to evaluate the effects of soil solarization and cabbage amendment on the survival of *P. nicotianae* and *P. capsici* in North Florida.

Materials and Methods

Characterization of the Solarization Sites

Two sites were selected for solarization in 1994 in commercial tomato production fields where control of soilborne diseases had been achieved previously through the use of a preplant application of methyl bromide plus chloropicrin. Site 1 was in the Naples Tomato Growers-Gargiulo farm number 4 in Decatur County, South Georgia. Site 2 was at the John Allen Smith farm located in Gadsden County, North Florida. In 1995, one experiment was conducted at the North Florida Research and Educational Center in Gadsden County (site 3, test 1) on soils that had been weed fallow for several years. In 1996, two experiments were conducted at the same center (site 3, tests 2 and 3) but in a different field. The soil at all locations consisted of Orangeburg or Tifton loamy fine sands (Typic Kandiudult: silicaceous, thermic), with pH values ranging from 4.7 to 6.6 (Table 2.1); these soils contained moderate amounts of kaolinitic clay and low amounts of organic matter.

Table 2.1. Characteristics of soils at the time of solarization.

Site	Location	pH	Soil water content[v] (-kPa)	Organic matter (%)	Percent sand-silt-clay	Soil class
1	NTG - Georgia[w]	6.6	5	1.1	84.0-6.0-10.0	Kandiudult[z]
2	JAS - Florida[x]	5.1	15	0.5	88.5-4.5-7.0	Kandiudult
3, test 1	NFREC - Quincy[y]	4.7	25	1.5	88.5-4.0-7.5	Kandiudult
3, test 2	NFREC - Quincy	5.0	5	1.0	80.4-11.1-8.5	Kandiudult
3, test 3	NFREC - Quincy	5.0	50	1.2	82.8-10.0-7.2	Kandiudult

[v] Estimated by the gravimetric method with oven drying, prior to initiation of the experiment, and transformed to matric potential (Appendix B).
[w] NTG - Georgia = Naples Tomato Growers-Gargiulo farm number 4 in Decatur County, Georgia.
[x] JAS - Florida = John Allen Smith farm located in Gadsden County, Florida.
[y] NFREC - Quincy = North Florida Research and Educational Center in Gadsden County.
[z] Ultisol with profiles similar to paleudults but containing higher amounts of kaolinitic clay.

Inoculum Production

Inoculum of *P. nicotianae* was produced by inoculating a 250-ml flask containing 20 g of wheat seeds and 30 ml of deionized water (autoclaved twice at a 24-hr interval) with four, 5-mm agar plugs of actively growing mycelium from a 4- to 7-day-old V8-juice-agar plate containing isolate Pn21. The flask was incubated at 25°C in the dark for 1 month and was shaken twice a week to ensure uniform growth of the isolate. Oospores of *P. capsici* were produced by inoculating a similarly prepared 250-ml flask of wheat seeds with three, 5-mm agar plugs of actively growing mycelium of each of two isolates of compatible mating types (Cp25 and Cp26). Isolate Pn21 was originally isolated from periwinkle, and isolates Cp25 and Cp26 were isolated from watermelon in South Florida; all isolates were maintained in the collection of *Phytophthora* spp. of the Plant Pathology Department of the University of Florida, Gainesville. The flask was incubated at 25°C in the dark for 2 months and was shaken twice a week to ensure uniform growth of both isolates on the substratum and to allow for maximum contact between the two mating types.

Inoculum of *P. nicotianae* or *P. capsici* was incorporated into the soil by mixing 200 mg of shredded, infested wheat seeds with 3 grams of soil. Nonpasteurized soils from sites 1 and 2 were used in 1994, and the soils were infested 2 days before the samples were taken to the field. Pasteurized soil from site 3, test 1, was infested with the inoculum 2 days before the samples were taken to the field in 1995. To encourage augmented formation of resting structures in the two experiments in 1996, pasteurized soil from site 1 was infested with either *P. nicotianae* and incubated at 18°C for 10 days, or with *P. capsici* and incubated at 25°C for 10 days, before its placement in the field tests. In all tests, each 3-g sample was enclosed in a 25-cm^2 nylon envelope (3-μm-pore size; Versapor 3000, Gelman Sciences, Inc.), and the envelopes were buried in the soil at depths of 10 and 25 cm just before application of the solarization treatments.

The survival of the spores at the end of the solarization experiments was determined by plating infested soil from the envelopes on a medium (PARPH) selective for pythiaceous fungi. The medium consisted of 17 g of cornmeal agar (Difco) in 1 liter of deionized water amended with 5 mg of pimaricin, 250 mg of ampicillin, 10 mg of rifampicin, 100 mg of pentachloronitrobenzene, and 50 mg of hymexazol (Mitchell and Kannwischer-Mitchell, 1992). In 1995 hymexazol was omitted from the selective medium.

The Solarization Experiments

Six solarization treatments with four replications each were selected for study at site 1, which consisted of an 18-treatment randomized complete block experiment on solarization and fumigation (Chellemi *et al.*, 1997). The selected treatments included a 30-µm-thick, clear, gas impermeable plastic film (Bromotec film, Lawson Mardon Packaging, United Kingdom); a 30-µm-thick, coextruded white-on-black, low density polyethylene film (Edison Plastics, Lee Hall, VA); and each of the polyethylene films with or without cabbage residue (*Brassica oleracea* var. *capitata* L. cv. Constanza) incorporated into the soil at a rate of 80 tons/ha. Controls consisted of a nontarped treatment and a tarped treatment (white-on-black film) fumigated with a 67:33 formulation of methyl bromide plus chloropicrin at 39.2 g/m^2.

At site 2, the six treatments selected for solarization from an 18-treatment randomized complete block experiment with four replications (Chellemi *et al.*, 1997) included a 30-µm-thick, clear, low density polyethylene film (Polydak film, Polyon Barkai Ltd., Kibutz Barkai, Israel); a 30-µm-thick, clear, gas impermeable plastic film (Bromotec film) ; and each of the polyethylene films with or without cabbage residue incorporated into the soil at a rate of 66 tons/ha. Controls consisted of a nontarped

treatment and a tarped treatment (white-on-black film) fumigated with a 67:33 formulation of methyl bromide plus chloropicrin at 39.2 g/m^2.

For test 1 at site 3, the eight treatments selected for solarization from an 15-treatment randomized complete block experiment with four replications (Chellemi *et al.*, 1997) included a 25-µm-thick, clear, low density polyethylene film (Polydak film); a 30-µm-thick, clear, gas impermeable plastic film (Bromotec film); a 30-µm-thick, coextruded white-on-black, low density polyethylene film (Edison Plastics); and each of the polyethylene films with or without cabbage residue incorporated into the soil at a rate of 68 tons/ha. Controls consisted of a nontarped treatment and a tarped treatment (white-on-black film) fumigated with a 67:33 formulation of methyl bromide plus chloropicrin at 39.2 g/m^2.

For tests 2 and 3 at site 3, six treatments were arranged in a randomized complete block design with four replications. Soil treatments included a 30-µm-thick, clear, gas impermeable plastic film (Bromotec film); a 30-µm-thick, coextruded white-on-black, low density polyethylene film (Edison Plastics); and each of the polyethylene films with or without cabbage residue incorporated into the soil at a rate of 89 tons/ha for test 2, and 81 tons/ha for test 3. Controls consisted of a nontarped treatment and a tarped treatment (white-on-black film) fumigated with a 67:33 formulation of methyl bromide plus chloropicrin at 39.2 g/m^2.

At sites 1 and 2 and for test 1 at site 3, each replicate plot consisted of one raised, 0.20-m X 0.90-m X 20-m bed, prepared according to standard commercial production practices; row orientation was north/south. Preplant fertilizer was broadcast into the beds at 212 kg of N, 65 kg of P, and 212 kg of K/ha, and drip irrigation tubing was placed 5 cm beneath the soil on sites 1 and 2. At site 3 for test 1, fertilizer was applied at 196 kg of N, 26 kg of P, and 163 kg of K/ha. Drip irrigation tubing was laid on the surface of the bed prior to covering with plastic. At site 3 for tests 2 and 3, each replicate plot consisted of one raised, 0.20-m X 0.90-m X 4.0-m bed prepared according to standard commercial

production practices; row orientation was north/south. No fertilizer was applied and no irrigation tubing was placed on the beds.

Cabbage was grown and harvested in the plots at sites 1 and 2, and the residue was spread over the plots on 23 and 24 May 1994. Beds were prepared, the nylon envelopes containing the inoculum were placed in the soil, and fumigant and plastic were applied on 3 June 1994 at site 1. The solarization period was terminated on 22 July 1994, after 49 days, and the samples were removed for determination of survival. At site 2, beds were prepared, the nylon envelopes containing the inoculum were buried, and fumigant and plastic were applied on 15 June 1994; the solarization period was terminated on 2 August 1994, after 48 days, when the samples were removed. For the first test at site 3, cabbage was grown in plots near the experimental plots, harvested, and the head and wrapper leaves incorporated into the plots on 19 May 1995. Beds were prepared, the inoculum bags were buried, and fumigant and plastic applied on 26 May 1995. The solarization period was terminated on 20 July 1995, after 55 days, when the samples were removed. For tests 2 and 3 at site 3, cabbage was harvested from a commercial field in Hastings, FL; head and wrapper leaves were incorporated into the plots on 12 June for test 2, and on 19 June 1996 for test 3. Beds were prepared, two sets of nylon envelopes containing the inoculum were placed in each plot, and fumigant and plastic were applied on 21 June and 28 June 1996, respectively. The solarization period was terminated with the removal of the last samples on 6 and 13 August 1996, after 45 days, for tests 2 and 3, respectively.

Daily ambient temperature data were obtained from a weather station at the North Florida Research and Educational Center, in Quincy, located approximately 25 km from sites 1 and 2, while daily precipitation amounts were recorded at each site. In 1994, soil temperatures were monitored in site 2 at depths of 10 and 25 cm using thermocouple sensors connected to an electronic data logger (Omnidata International, Logan, UT). For all three tests at site 3, daily rainfall and ambient temperature data were recorded at the

site. For test 1 at site 3, hourly temperature changes were monitored for 33 days at an external test location approximately 500 m from test 1; however, temperatures were still recorded at 10 and 25 cm depths in the soil of the nontarped and the clear, gas impermeable treatments with thermocouple sensors connected to an electronic data logger (Campbell Scientific, Logan, UT). In 1996, temperature changes were monitored in test 2, at 10 and 25 cm depths in the soil.

Statistical Analysis

All data of survival of each pathogen were log-transformed (ln[ppg+1]) prior to analysis of variance. Analysis of variance was performed with PROC MIXED of SAS (SAS Institute, Cary, NC; release 6.11 for personal computers), considering that the environmental effects on the inoculum due to the depth factor could be correlated. Survival of *P. nicotianae* and *P. capsici* was compared using Tukey's Honestly Significant Difference procedure.

Results

Environmental Conditions

Environmental conditions varied from year to year, with 1994 being characterized by above average precipitation and below average temperatures (Figure 2.1). In 1995 both precipitation and temperatures were normal, and in 1996 precipitation was below average and temperatures were normal. The heating process of the soil was interrupted by precipitation during 61% of the days of the solarization period at site 1; 65% of the days at site 2; 30% of the days in test 1 at site 3; 42% of the days in test 2 at site 3; and 47% of the days in test 3 at site 3 (Table 2.2).

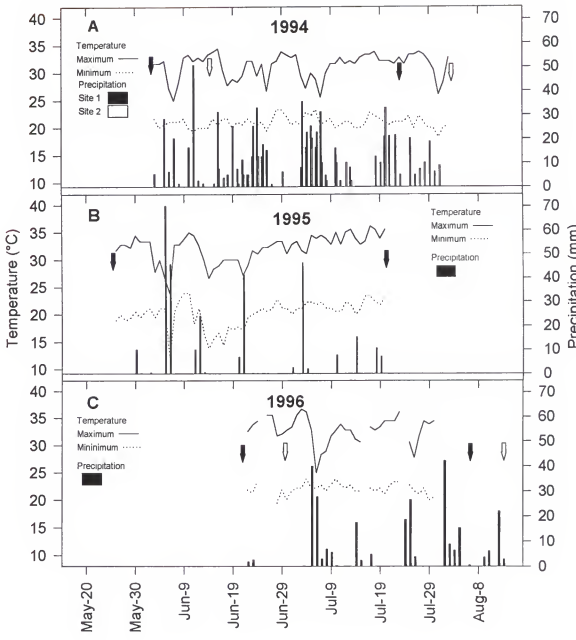

Figure 2.1: Maximum and minimum air temperature and precipitation recorded at each site during the solarization period, from 1994 to 1996. A. Solid arrows (⬇) indicate beginning and end of solarization at Site 1; open arrows (⇩) indicate beginning and end of solarization at Site 2. B. Solid arrows (⬇) indicate beginning and end of solarization in test 1 at Site 3. C. Solid arrows (⬇) indicate beginning and end of solarization in test 2 at Site 3; open arrows (⇩) indicate beginning and end of solarization in test 3 at Site 3.

Table 2.2. Ambient weather conditions during solarization studies in 1994, 1995, and 1996.

Site	Location	Year	Precipitation (mm)	Number of days		
				Solarization period	Precipitation occurred	Ambient temperature ≥35
1	Georgia[x]	1994	460	49	30	0
2	Florida[y]	1994	384	48	31	0
3, test 1	NFREC - Quincy[z]	1995	301	55	17	6
3, test 2	NFREC - Quincy	1996	232	45	19	6
3, test 3	NFREC - Quincy	1996	264	45	21	4

[x] Georgia = Naples Tomato Growers-Gargiulo farm number 4 in Decatur County, Georgia.
[y] Florida = John Allen Smith farm located in Gadsden County, Florida.
[z] NFREC - Quincy = North Florida Research and Educational Center in Gadsden County.

Soil moisture conditions at the beginning of the solarization treatments varied from experiment to experiment and ranged from -5 to -50 kPa (Table 2.1). Even though there were several rain events during the solarization period in all experiments, it is possible that soil moisture was not replenished under the plastic films used due to the structure of the sandy soils studied; the high proportion of large size pores in these soils does not favor the lateral movement of water.

During the 48 days of solarization at site 2, there were 2 days in which temperatures exceeded 41°C at the 10-cm soil depth in the nontarped treatment; 41°C was exceeded on 15 days in the clear plastic treatment and on 20 days in the gas impermeable treatment (Table 2.3). Temperatures exceeded 44°C at the 10-cm depth on 9 days under the clear plastic and on 13 days under the gas impermeable film. Temperatures were above 47°C for 1 day and 3 days under the clear and the gas impermeable films, respectively. At the 25-cm depth, temperatures never reached the 41°C threshold under any of the treatments. Maximum temperatures attained in site 2 at the 10-cm depth under nontarped soil, clear low density polyethylene plastic, and clear, gas impermeable plastic were 41.5°, 47.3°, and 48.5°C, respectively. At the 25-cm depth, maximum temperatures were 34.6°, 37.9°, and 38°C under nontarped soil, clear low density polyethylene plastic, and clear, gas impermeable plastic, respectively. The average soil temperature change during the day is illustrated in Figure 2.2.A. Temperature was not monitored in site 1.

At site 3 for test 1, temperatures above 41°C were recorded at the 10-cm depth under the clear low density polyethylene plastic on 30 days, and under the nontarped treatment on 5 days; 44°C was reached on 20 days and 47°C was reached on 6 days during the 33 days over which temperatures were monitored under the clear low density polyethylene film. At the 25-cm depth, temperatures never reached the 41°C threshold (Table 2.3). Maximum soil temperatures under nontarped soil and clear low density polyethylene plastic at the 10-cm depth were 42.4° and 48.7°C, respectively; at the 25-cm

Table 2.3. Temperature profiles during soil solarization at site 2 and for tests 1, 2, and 3 at site 3.

Site[v]/Test	Depth	Nontarped[w]			White-on-black			Clear LDPE			Gas Impermeable		
	(cm)	41°C	44°C	47°C	41°C	44°C	47°C	41°C	44°C	47°C	41°C	44°C	47°C
		number of days above threshold											
Site 2	10	2	0	0	nt[x]	nt	nt	15[y]	9	1	20	13	3
	25	0	0	0	nt	nt	nt	0	0	0	0	0	0
Site 3/1	10	5	0	0	nm[z]	nm	nm	30	20	6	nm	nm	nm
	25	0	0	0	nm	nm	nm	0	0	0	nm	nm	nm
Site 3/2	10	1	0	0	0	0	0	nt	nt	nt	32	24	10
	25	0	0	0	0	0	0	nt	nt	nt	1	0	0
Site 3/3	10	0	0	0	0	0	0	nt	nt	nt	30	22	8
	25	0	0	0	0	0	0	nt	nt	nt	1	0	0
		number of hours above threshold											
Site 2	10	4	0	0	nt	nt	nt	63	25	2	82	42	7
	25	0	0	0	nt	nt	nt	0	0	0	0	0	0
Site 3/1	10	10	0	0	nm	nm	nm	161	84	14	nm	nm	nm
	25	0	0	0	nm	nm	nm	0	0	0	nm	nm	nm
Site 3/2	10	1	0	0	0	0	0	nt	nt	nt	225	125	24
	25	0	0	0	0	0	0	nt	nt	nt	4	0	0
Site 3/3	10	0	0	0	0	0	0	nt	nt	nt	203	114	21
	25	0	0	0	0	0	0	nt	nt	nt	4	0	0

[v] Site 2 = John Allen Smith farm located in Gadsden County, Florida (solarized for 48 days in 1994); Site 3 = North Florida Research and Educational Center in Gadsden County (solarized for 55 days in test 1, and for 45 days in tests 2 and 3).

[w] Temperatures were measured in the untreated soil (nontarped); under a 30-µm-thick, coextruded white-on-black, low density polyethylene film (White-on-black); under a 30-µm-thick, clear, low density polyethylene film (Clear LDPE); and under a 30-µm-thick, clear, gas impermeable plastic film (Gas Impermeable).

[x] nt= not tested.

[y] Temperatures measured during the first 42 days of solarization.

[z] nm = not measured.

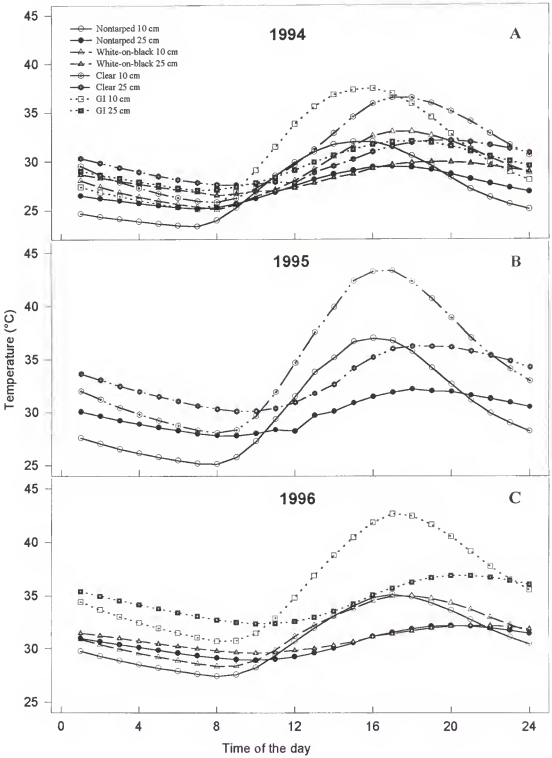

Figure 2.2: Average temperature change during a 24 hour period at 10 and 25 cm of depth. Temperatures were measured in the untreated soil (nontarped); under the white-on-black, low density polyethylene film (White-on-black); under the clear, low density polyethylene film (Clear);and under the clear, gas impermeable plastic film (GI). **A**. Site 2 (John Allen Smith farm, Gadsden County); **B**. Site 3 (North Florida Research and Educational Center in Gadsden County) test 1; **C**. Site 3 test 2.

depth maximum temperatures were 35.1° and 40.3°C. The average soil temperature change during the day is illustrated in Figure 2.2.B.

During the 45 days of solarization at site 3 for test 2, maximum temperatures attained at the 10-cm depth under nontarped soil, white-on-black plastic, and clear, gas impermeable plastic were 41.1°, 39.0° and 49.1°C, respectively. At the 25-cm depth, maximum temperatures were 37.4°, 37.5°, and 41.4°C under nontarped soil, white-on-black plastic, and clear, gas impermeable plastic, respectively. Under the clear, gas impermeable plastic, at the 10-cm depth, temperatures were above 41°C for 32 days, above 44°C for 24 days, and above 47°C for 10 days; while at the 25-cm depth, 41°C was reached on only 1 day. Temperatures in the nontarped treatment reached 41°C only once during the solarization period, and under the white-on-black plastic temperatures never reached that threshold. The average temperature change during the day is illustrated in Figure 2.2.C.

Soil temperature changes for test 3 at site 3 were monitored using thermocouples placed in test 2 at site 3, which was located a few meters away. Maximum temperatures attained at the 10-cm depth under nontarped soil, white-on-black plastic, and clear, gas impermeable plastic were 41.1°, 44.2° and 49.1°C, respectively. At the 25-cm depth, maximum temperatures were 37.4°, 37.5°, and 41.4°C under nontarped soil, white-on-black plastic, and clear, gas impermeable plastic, respectively. Under the clear, gas impermeable plastic, at the 10-cm depth, temperatures were above 41°C for 30 days, above 44°C for 22 days, and above 47°C for 8 days. Temperatures never reached these thresholds at the 25-cm depth. Temperatures in the nontarped treatment and in the white-on-black plastic never reached the 41°C threshold.

The highest accumulation of hours in which soil temperatures were greater than 41°, 44° and 47°C was observed at the 10-cm depth in test 2 at site 3, followed by test 3 at site 3. The lowest accumulation of hours was observed at site 2 (Table 2.3). At site 2 it was possible to compare the accumulation of hours above the given thresholds under the

solarization films. Under the gas impermeable film, temperatures were above 41°, 44° and 47°C for 82, 42 and 7 hours, respectively; while under the clear low density polyethylene plastic the accumulation was of 63, 25, and 2 hours, respectively. In the nontarped treatment and at the 25-cm depth of all treatments, temperatures never reached the 44°C threshold.

Survival of *Phytophthora nicotianae*

Recovery of *P. nicotianae* varied from site to site and from year to year. At site 1 in 1994, no propagules were recovered from the nontarped or the methyl bromide treatments, or from the 10-cm depth in the gas impermeable treatment, with or without cabbage, after 49 days of solarization (Table 2.4). The highest survival was observed under the white-on-black polyethylene film at 10 cm of depth with or without cabbage amendment. At the 25-cm depth under the gas impermeable film, propagules were recovered at the same levels as in the white-on-black film treatment.

At site 2 in 1994, *Burkholderia cepacia*, a contaminant from the soil, covered the selective medium plates in such a way that very few propagules of *P. nicotianae* were recovered, and only from soil under the clear low density polyethylene at 10-cm of depth (data not shown).

The omission of hymexazol in the selective medium for the determination of survival of *P. nicotianae* from site 3 for test 1, made it impossible to recover the pathogen from the soil because other microorganisms, especially *Pythium* spp., colonized the organic substratum (shredded wheat seeds) and may have inhibited *P. nicotianae* in the soil or on the dilution plates.

Propagules of *P. nicotianae* were recovered from all treatments in test 2 at site 3, except from soil treated with methyl bromide; however, there were no statistically significant differences among the treatments (Table 2.4).

Table 2.4. Survival of *Phytophthora nicotianae* after soil solarization.

Treatment[u]	Depth (cm)	Propagules/g of soil in 1994 Site 1[v]	Propagules/g of soil in 1996 Site 3[w]- test 2	Propagules/g of soil in 1996 Site 3 - test 3
Gas impermeable	10	0.0[x] a[y]	0.4a	4.4a
Gas impermeable	25	39.1 abc	2.1a	202.3b
Gas impermeable + Cabbage	10	0.0 a	0.9a	1.0a
Gas impermeable + Cabbage	25	7.2 ab	2.3a	362.0b
White-on-black	10	380.5 c	4.0a	733.9b
White-on-black	25	12.9 abc	0.9a	697.8b
White-on-black + Cabbage	10	439.1 c	14.8a	825.3b
White-on-black + Cabbage	25	44.9 bc	12.3a	749.5b
Methyl bromide	10	0.0 a	0.0a	0.0a
Methyl bromide	25	0.0 a	0.0a	0.0a
Non-tarped	10	0.0 a	14.9a	700.7b
Non-tarped	25	0.0 a	4.5a	687.3b

[u] Treatments consisted of untreated soil (Non-tarped); soil fumigated with methyl bromide (Methyl bromide); soil solarized under a 30-µm-thick, coextruded white-on-black, low density polyethylene film (White-on-black); or soil solarized under a 30-µm-thick, clear, gas impermeable plastic film (Gas Impermeable). Soil was either nonamended or amended with cabbage (+ Cabbage) at 80, 89, and 81 tons/ha at site 1, and for tests 2 and 3 at site 3, respectively.

[v] Site 1 = Naples Tomato Growers-Gargiulo farm number 4 in Decatur County, Georgia (solarized for 49 days).

[w] Site 3 = North Florida Research and Educational Center in Gadsden County (solarized for 45 days).

[x] Weighted means ([Exp {mean}]-1).

[y] Main effect means followed by the same letter do not differ according to Tukey's Honestly Significant Difference procedure ($P \leq 0.05$); data were transformed to ln(ppg+1) for analysis.

[z] nt = not tested.

Trends similar to those observed in site 1 were observed in test 3 at site 3 (Table 2.4). Only methyl bromide eliminated the pathogen from the soil. The gas impermeable film was the next best treatment at the 10-cm depth, regardless of the cabbage amendment, for the reduction of the population of the pathogen; however, at the 25-cm depth the gas impermeable film was not effective, and more than 200 propagules survived in each gram of soil. These values were not statistically different from those observed under the white-on-black film, with or without cabbage amendment, or in the nontarped treatment. Survival in the nontarped treatments in tests 2 and 3, in contrast to no survival in Site 1 could have been due to the change in the procedure for soil infestation from that used for site 1; chlamydospores were already present at the beginning of the experiments in the soil prepared for tests 2 and 3, but not for test 1.

Samples removed after 17 days of solarization in tests 2 and 3 at site 3 demonstrated that the pathogen was present in the samples, except in the methyl bromide treatment, and that the populations declined with time of exposure to the solarization process (Table 2.5). Increases in population were observed in test 3, mostly at the 25-cm depth, in the nontarped, gas impermeable, and white-on-black treatments after the 45 days of solarization. Statistically significant differences were observed at the 10-cm depth between the two sampling dates under the gas impermeable film; the population of *P. nicotianae* dropped to levels comparable to those in the methyl bromide treatment.

Survival of *Phytophthora capsici*

Oospores of *Phytophthora capsici* do not germinate readily *in vitro*, and this was clearly noted during the field experiments. Although the presence of oospores was verified in the inoculum before the soil was infested, there was not a corresponding high recovery

Table 2.5. The effect of time of exposure to solarization on the survival of *Phytophthora nicotianae* at Site 3.

Treatment[x]	Depth	Propagules per gram of soil			
		Test 2		Test 3	
	(cm)	17 days	45 days	17 days	45 days
Gas Impermeable	10	2.0[y] a[z]	0.4a	544.6b	4.4a
Gas Impermeable	25	4.1 a	2.1a	168.0b	202.3b
Gas Impermeable + Cabbage	10	2.7 a	0.9a	52.5b	1.0a
Gas Impermeable + Cabbage	25	0.7 a	2.3a	491.3b	362.0b
White-on-black	10	22.8 a	4.0a	196.6b	733.9b
White-on-black	25	16.3 a	0.9a	186.4b	697.8b
White-on-black + Cabbage	10	101.9 a	14.8a	830.5b	825.3b
White-on-black + Cabbage	25	25.0 a	12.3a	608.3b	479.5b
Methyl Bromide	10	0.0 a	0.0a	0.0a	0.0a
Methyl Bromide	25	0.0 a	0.0a	0.0a	0.0a
Nontarped	10	21.6 a	14.9a	1138.7b	700.7b
Nontarped	25	6.2 a	4.5a	311.8b	687.6b

[x] Treatments consisted of untreated soil (Nontarped); soil fumigated with methyl bromide plus chloropicrin at 39.2 g/m^2 (Methyl bromide); soil solarized under a 30-µm-thick, coextruded white-on-black, low density polyethylene film (White-on-black); or soil solarized under a 30-µm-thick, clear, gas impermeable plastic film (Gas Impermeable). Soil was either nonamended or amended with cabbage (+ Cabbage) at 89 and 81 tons/ha for tests 2 and 3, respectively.

[y] Weighted means ([Exp {mean}]-1).

[z] Main effect means at each date for each test followed by the same letter do not differ according to Tukey's Honestly Significant Difference procedure ($P \leq 0.05$); data transformed to (ln[ppg+1]) prior to analysis.

of the pathogen, either before or after the solarization treatments. Recovery of the pathogen on the selective medium was erratic.

At site 1, no propagules were recovered at 10-cm depth after treatment with methyl bromide, the gas impermeable plastic, or the gas impermeable plastic combined with cabbage (Table 2.6). Low levels of survival at the 25-cm depth under the methyl bromide treatment, the gas impermeable plastic, and the gas impermeable plastic combined with cabbage were not statistically different from the same treatments at the 10-cm depth. The highest survival was observed under the white-on-black plastic at the 10-cm depth, regardless of cabbage amendment, and in the nontarped treatment.

At site 2, no statistical differences were observed in the survival of *P. capsici* under the clear low density polyethylene film, clear low density polyethylene film plus cabbage, and the gas impermeable plastic at the 10-cm depth, or under the methyl bromide and the gas impermeable plastic combined with cabbage at both depths (Table 2.6). Survival was highest in the nontarped treatment at both depths, and populations were not significantly different from those at the 25-cm depth in all other treatments, except treatments with methyl bromide and gas impermeable plastic combined with cabbage.

The lack of hymexazol in the selective medium for the determination of survival of *P. capsici* from test 1 at site 3 made it impossible to recover the pathogen from the soil because other microorganisms colonized the organic substratum (shredded wheat seeds) and grew faster than the pathogen on the dilution plates. Not even the addition of 100 mg of lecithin per liter of medium to stimulate the germination of oospores of the pathogen could compensate for the lack of hymexazol.

Fewer oospores were observed in the inoculum used for tests 2 and 3 at site 3, than in previous experiments, and very few colonies were observed on the selective medium at the end of the experiments. No valid analysis could be performed and the data are not presented.

Table 2.6. Survival of *Phytophthora capsici* after soil solarization in 1994.

Treatment[u]	Depth (cm)	Site 1[v] (ppg)	Site 2[w] (ppg)
Gas Impermeable	10	0.0[x] a[y]	0.0a
Gas Impermeable	25	1.5 ab	7.0ab
Gas Impermeable + Cabbage	10	0.0 a	0.4a
Gas Impermeable + Cabbage	25	1.2 ab	0.5a
Clear LDPE	10	nt[z]	0.0a
Clear LDPE	25	nt	11.2ab
Clear LDPE + Cabbage	10	nt	0.2a
Clear LDPE + Cabbage	25	nt	7.9ab
White-on-black	10	84.6 c	nt
White-on-black	25	47.4 c	nt
White-on-black + Cabbage	10	70.8 c	nt
White-on-black + Cabbage	25	16.3 bc	nt
Methyl Bromide	10	0.0 a	0.2a
Methyl Bromide	25	1.1 ab	0.9a
Non-tarped	10	33.4 c	33.9b
Non-tarped	25	63.0 c	25.6b

[u] Treatments consisted of untreated soil (Non-tarped); soil fumigated with methyl bromide plus chloropicrin at 39.2 g/m^2 (Methyl bromide); soil solarized under a 30-µm-thick, coextruded white-on-black, low density polyethylene film (White-on-black); or soil solarized under a 30-µm-thick, clear, low density polyethylene film (Clear LDPE); or a 30-µm-thick, clear, gas impermeable plastic film (Gas Impermeable). Soil was either nonamended or amended with cabbage (+ Cabbage) at 80 and 68 tons/ha at sites 1 and 2, respectively.

[v] Site 1 = Naples Tomato Growers-Gargiulo farm number 4 in Decatur County, Georgia (49 days of solarization).

[w] Site 2 = John Allen Smith farm located in Gadsden County, Florida (48 days of solarization).

[x] Weighted means ([Exp {mean}]-1).

[y] Main effect means followed by the same letter do not differ according to Tukey's Honestly Significant Difference procedure ($P \leq 0.05$); data were transformed to ln(ppg+1) for analysis.

[z] nt = not tested.

Discussion

Temperature Changes and Soil Moisture Content

Soil solarization is a pasteurization process dependent on solar energy to heat the soil and kill pathogens. Effective pasteurization occurs when soil sustains temperatures that are lethal to the fungal propagules infesting the soil. In arid climates, soil temperatures between 48° and 54°C have been reported as effective for the control of *Rhizoctonia solani*, *Pythium* spp., *Thielaviopsis basicola* (Pullman *et al.*, 1979), *Verticillium dahliae* (Grinstein *et al.*, 1979b; Pullman *et al.*, 1979), *Pyrenochaeta terrestris*, *Fusarium spp.* (Katan *et al.*, 1980) and *Sclerotium rolfsii* (Grinstein *et al.*, 1979a). However, in climates where cloud cover and rainfall interrupt the heating process of the soil, maximum temperatures recorded were below 50°C (Chellemi *et al.*, 1994).

Temperature changes were monitored in site 2 for the experiments in sites 1 and 2, and in test 2 at site 3 for both tests 2 and 3. Inferences from one site in relation to the other have to be made with caution since soil color, proportion of sand, silt and clay content, and soil moisture were different. Each one of these factors can affect the temperature accumulation in soil, and when taken together could cause significant deviations in temperatures from the patterns observed at the site where temperatures were actually measured. These differences may hold for sites that are several kilometers apart (sites 1 and 2), as well as for sites a few meters apart, as was the case in site 3, between tests 2 and 3. Because tests 2 and 3 at site 3 were only a few meters apart, with the same type of soil, any differences in temperature could be explained best by the change in moisture content. As shown by Mahrer *et al.* (1984), the higher the water content of the soil, the higher the maximum temperature will be.

Survival of *Phytophthora nicotianae*

Phytophthora nicotianae is believed to survive in root fragments and organic matter in the soil or as chlamydospores free in the soil (Erwin and Ribeiro, 1996). Infested wheat seeds were selected for inoculum to favor the formation of chlamydospores in soil and thus simulate natural spore formation for field survival. It is possible that lower temperatures during the first days of solarization allowed the formation of chlamydospores in the soil, and, therefore, a higher recovery was observed at the end of some experiments. Soil moisture also may have contributed to the variability in recovery of *P. nicotianae* from test to test and site to site. In test 2, the plastic treatments were laid down after a week of frequent rains, with soil moisture very uniform throughout the soil profile. In test 3, the week preceding the application of the plastic treatments was hot and dry, and irrigation was required to provide adequate moisture for the solarization. These factors were reflected in the results observed for the tests, especially in test 3, where higher numbers of surviving propagules were observed at lower depths, possibly indicating that temperatures did not rise as much as in test 2 due to the lack of moisture. According to Mahrer *et al.* (1984) temperature maxima of the soil increase with increasing soil moisture content. A 10-fold difference in the water status was observed between tests 2 and 3, which may have led to differences in survival of *P. nicotianae* at lower depths.

The absence of chlamydospores in the wheat seeds at the time of preparation of the inoculum and a subsequent lack of survival of *P. nicotianae* in the nontarped treatments in 1994 and 1995 prompted a search for methods that would augment the production of this type of inoculum. Ioannou and Grogan (1985) indicated that chlamydospores were formed abundantly when mycelial mats in liquid cultures were exposed to an environment containing 10% CO_2 and 2% O_2. This procedure was evaluated for the wheat seed inoculum, but increased chlamydospore production did not

occur (Appendix A). However, when soil was infested and incubated at 18° or 25°C, large numbers of chlamydospores were formed (Appendix A). Since the highest number of chlamydospores was observed in the pasteurized soil from site 1 after incubation at 18°C for 10 days, inoculum was prepared in 1996 by infesting microwaved soil from site 1 and incubating it at 18°C for 10 days before burial in the field.

The clear, low density polyethylene and the gas impermeable films were effective in reducing populations of *P. nicotianae* at the 10-cm depth. At this depth control was similar to that achieved by fumigation with methyl bromide. However, at the 25-cm depth, no control was observed, and the populations recovered on the dilution plates were similar to those in the nontarped or the white-on-black plastic controls. Clearly, solarization alone cannot be relied upon to control *P. nicotianae* in soil under conditions encountered in these tests. Chellemi *et al.* (1994) found that soil solarization reduced populations of *P. nicotianae* to undetectable levels at a depth of 15 cm; however, at the 25-cm depth, no differences in survival were observed between the solarized and the control treatments in two sites.

Survival of *Phytophthora capsici*

Although the same two isolates were used in all crosses to produce oospores for all tests, the numbers of oospores observed in wheat seeds were not consistent from year to year. Another problem associated with the evaluation of survival of *P. capsici* is the germination of the propagules in the selective medium. In preliminary studies it was found that approximately 1% of the oospores germinated in PARPH, which was confirmed by the work of Larkin *et al.* (1995). *Phytophthora capsici* survived better in the nontarped treatments and under the white-on-black plastic, regardless of the cabbage amendment. Control of the pathogen was most effective with methyl bromide, followed by the gas impermeable and clear low density polyethylene film at the 10-cm depth. In

1995 and 1996, fewer oospores were produced in the wheat seeds and the recovery of propagules after solarization was inconsistent, which precluded analysis of the results.

Effect of Cabbage Amendment

Cabbage did not affect the survival of either pathogen. This finding is contrary to that observed by other researchers (Keinath, 1996; Ramirez-Villapudua and Munnecke, 1987, 1988). It is possible that the differences observed are due to the preparation of the cabbage amendment and its incorporation into the soil. For all of the field plots, cabbage was either chopped with a machete (1994) or shredded in a shredder (1995 and 1996). In either case, the fragments were relatively large, and incorporation was done by disking the residue into the soil at the same time that the raised beds were being prepared, which may have led to an uneven distribution of the amendment in the soil profile. In contrast, Ramirez-Villapudua and Munnecke (1988) air dried the cabbage and ground it with a Wiley mill to a fine powder. This residue was incorporated into small amounts of soil, which were placed in plastic bags to be solarized. Further work by these authors (Ramirez-Villapudua and Munnecke, 1987) indicated that populations *of Fusarium oxysporum* f.sp. *conglutinans* were reduced by the incorporation of cabbage into the soil at rates that were higher than those used in the experiments reported here. Keinath (1996) found that gummy stem blight of watermelon was reduced in areas that received cabbage amendment and solarization; however, no information about the amount of residue incorporated into the soil was given. It may be that the effectiveness of cabbage as a soil amendment is influenced by the drying process, and, during the experiments reported here, rainfall during the drying time may have had a detrimental effect.

CHAPTER 3
THERMAL INACTIVATION OF *PHYTOPHTHORA NICOTIANAE*

Introduction

Studies of thermal inactivation of plant pathogens can yield important information on survival of specific types of propagules, and, using simulated conditions, can provide information for the estimation of time constraints for effective soil solarization. Juarez-Palacios *et al.* (1991) found that determination of the heat sensitivity of isolates of *Phytophthora* spp. in the laboratory closely reflected their inactivation in solarized soil and indicated the possible use of soil solarization for management of those pathogens. These authors found that a high-temperature isolate of *P. megasperma* survived exposure for 30 minutes at 45°C in a heat sensitivity study, and that leaf discs used as bait were infected by the pathogen after solarization for 4 weeks; however, *P. cinnamomi* and a low-temperature isolate of *P. megasperma* did not survive exposure to 45°C for 20 minutes. In similar studies, Bollen (1985) observed that oospores of *P. capsici* were more thermotolerant than mycelium from either mating type used to produce the oospores. The temperature difference for tolerance was more than 5°C, with oospores surviving at 50°C for 30 minutes. However, the system used did not reflect field conditions, since the continuously flooded soil employed in the experiment would rarely be attained in any field. Bollen (1985) did not analyze the relationship of time to varying temperature, and the standard temperature of 50°C used in the laboratory tests is rarely obtained at soil depths below 10 cm. One interesting aspect of this study was the use of cucumber seedlings for a plant disease assay, which was more sensitive than soil dilution plating on

potato-dextrose agar (PDA, pH 5.6) for the detection of residual populations of the pathogen.

Pullman *et al.* (1981a) observed a logarithmic relationship between time and temperature in the inactivation of propagules of four soilborne plant pathogens. These authors found that the exposure time required to reduce the population by 90% (LD_{90}) for oospores of *Pythium ultimum* was 30 minutes at 50°C, 110 minutes at 47°C, 8 hours at 45°C, 42 hours at 42°C, 13 days at 38.5°C and 26 days at 37°C, respectively. This study was performed using soil moisture adjusted to field capacity, and it closely reflected common temperature profiles in soil solarization tests.

Studies of thermal inactivation can be complemented to simulate other conditions in the field, such as the addition of organic amendments to the soil prior to solarization (Gamliel and Stapleton, 1993a, 1993b; Ramirez-Villapudua and Munnecke, 1987, 1988). Cabbage residue associated with solarization was used successfully to control cabbage yellows, caused by *Fusarium oxysporum* f. sp. *conglutinans*, by Ramirez-Villapudua and Munnecke (1987, 1988). The addition of cabbage alone was only partially effective for the control of cabbage yellows, but, when cabbage residue was heated during solarization, there was significant control of the pathogen.

Due to the demands on time and equipment, thermal inactivation studies very seldom explore extensive combinations of both time and temperature ranges. Thus, the full benefits that can be derived from such experiments are often not realized. The objectives of this study were to evaluate the effects of both constant and pulsing temperatures, cabbage amendment, soil water matric potential, and three, pasteurized and nonpasteurized soils on the survival of *Phytophthora nicotianae*.

Materials and Methods

Production of Chlamydospore Inoculum of *Phytophthora nicotianae*

Chlamydospores of *Phytophthora nicotianae* were produced in V8 broth, as described by Mitchell and Kannwischer-Mitchell (1992). Four, 5-mm-diameter V8 juice-agar plugs of a culture with actively growing mycelium of isolate Pn21 were transferred to a 325-ml prescription bottle containing 25 ml of clarified V8 broth. After incubation at 25°C in the dark for 24 hours, the bottle was shaken vigorously to fragment the mycelial mats. The hyphal fragments adhering to the walls were resuspended by slowly rotating the bottle. The bottle was incubated horizontally as a stationary culture at 25°C for 6 days. One hundred milliliters of sterile deionized water were added to submerge the mycelial mat, and the culture was further incubated at 18°C, vertically, for a minimum of 3 weeks.

The mycelial mats were rinsed in deionized water on a 400-mesh sieve, transferred to a blender with enough water to make a slurry, and blended on high for 1 minute. The resulting slurry was ground about 30 times in a glass mortar and pestle and then subjected to two, 30-second cycles of sonication at 100 W (Model 450 Sonifier, Branson Ultrasonics Corporation, Danbury, CT 06810). The total number of chlamydospores in the suspension was estimated with a hemacytometer, and the suspension was immediately used to infest soil to a density of 500 chlamydospores per gram of soil.

Effect of Constant Temperature on the Inactivation of Chlamydospores of *Phytophthora nicotianae*

One-kilogram lots of moist soil from Site 1 (Naples Tomato Growers-Gargiulo farm number 4 in Decatur County, Georgia) were pasteurized in a microwave at 700 W

for 4 minutes in a plastic bag. After pasteurization soil moisture was adjusted to 6% (-15 kPa) with sterile water. Thirty grams of infested soil were dispensed into a test tube, which was loosely closed with a plastic cap to allow exchange of air.

A set of test tubes was placed in each of eight water baths held at each of the following constant temperatures with circulation heaters: 25°, 35°, 38°, 41°, 44°, 47°, 50° and 53°C. The time of exposure at each temperature varied from 5 to 120 minutes or from 2 to 480 hours. Three tubes were removed at each time interval and part of the soil (15 g) was diluted with soft agar (2.5 g of Difco agar per liter of deionized water) and plated on a medium selective for pythiaceous fungi within 24 hours. The selective medium (PARP) consisted of 17 g of cornmeal agar (Difco) in 1 liter of deionized water amended with 5 mg of pimaricin, 250 mg of ampicillin, 10 mg of rifampicin, and 100 mg of pentachloronitrobenzene (Mitchell and Kannwischer-Mitchell, 1992). The soil overlay was removed after 48 hours by gently washing the agar surface with tap water. The total number of colonies formed on PARP after 72 hours of incubation was recorded as an estimation of the number of chlamydospores surviving the heat treatment. The other part of the soil (15 g) was transferred to a Petri plate and covered with approximately 10 ml of sterile water to provide a thin layer of water over the soil. Three, 3-day-old tomato seedlings, cultivar solar set, were placed on the soil in each plate and incubated in a growth chamber at 27°C for 3 days. The seedlings were rinsed twice in sterile water, blotted dry and plated on PARP to evaluate the colonization of tissues by *P. nicotianae*. The number of seedlings with fungal colonization was recorded. The experiment with times of exposure ranging from 5 to 120 minutes was repeated once, while the experiment with times of exposure ranging from 2 to 480 hours was repeated twice.

The Effect of Cabbage Amendments on the Thermal Inactivation of Chlamydospores of *Phytophthora nicotianae*

In order to determine the effect of cabbage amendments on the inactivation of chlamydospores of *P. nicotianae*, experiments similar to the ones described previously were conducted, but temperatures were restricted to 35°, 38°, and 41°C, and the time was limited from 2 to 480 hours. Cabbage leaves (*Brassica oleracea* var. *capitata*) were air dried in a greenhouse and ground in a Wiley mill with a 20-mesh screen. Pasteurized soil was amended with dry, ground cabbage leaves at 0, 0.125, 0.25 or 0.5% (w/w). Preliminary work indicated that concentrations above 0.5% were phytotoxic to tomato seedlings. Thirty grams of amended soil were dispensed into a test tube, a disk of filter paper was laid on top of the soil, and then thirty grams of amended and infested soil were dispensed on the top of the first layer. The tube was sealed with a gas impermeable plastic film (Bromotec film, Lawson Mardon Packaging, United Kingdom), and a plastic cap was placed over the tube to reduce the exchange of air and trap the volatiles released by heating the cabbage amended soil.

The test tubes were placed in water baths held at constant temperatures with circulation heaters. Three tubes were removed at each time interval, and part of the soil (15 g) was diluted with soft agar and plated on PARP within 24 hours, as previously described. The soil overlay was removed after 48 hours by gently washing the agar surface with tap water, and the number of colonies was recorded. Fifteen grams of soil were transferred to Petri plates and covered with sterile water, and three, 3-day-old tomato seedlings were incubated on the soil slurry for 3 days. After incubation the seedlings were rinsed twice in sterile water, blotted dry, and plated on PARP. The number of colonized seedlings was recorded.

Another test was done to determine if the bottom layer was necessary to ensure adequate concentration of volatiles in the test tube. Soil infested with the pathogen was

dispensed into empty test tubes or into tubes containing a bottom layer of cabbage-amended, noninfested soil. All test tubes were placed in the water baths at constant temperatures, as described above, and incubated for 1 week. The tubes were removed from the water baths, and the number of propagules were estimated using procedures previously described. No significant effects of the soil layering were observed when the data were analyzed. For this reason all other tests were done with only a single layer of infested, cabbage-amended soil.

The Effect of Cycling Temperatures and Cabbage Amendments on the Thermal Inactivation of Chlamydospores of *Phytophthora nicotianae*

A more critical analysis of the effects of daily temperature fluctuation, as observed during soil solarization, on the survival of the pathogen was performed in this experiment. Two temperatures, representing average thresholds at 10 and 25 cm of depth, were selected, and the duration of the daily exposure at each temperature was determined by an analysis of published data from north Florida (Chellemi *et al.*, 1994). The daily regimes selected were 5 hours at 41°C and 8 hours at 35°C, with a baseline temperature of 25°C during the rest of the day. These temperatures were maintained with circulation heaters in water baths. After preliminary tests indicated a high percentage of the spores survived the heat treatment, a third temperature, 44°C for 1.5 hours daily, was added. The duration of the daily exposure at 44°C was based both on the results of previous tests with constant temperature and on the analysis of temperature profiles from field experiments in 1994 (Chellemi *et al.*, 1994).

Microwaved and infested soil was adjusted to a final moisture of 6% with sterile water. Half of the infested soil was amended with dry, ground cabbage leaves at 0.125% (w/w). Thirty grams of soil were dispensed into a test tube, which was sealed with a piece

of the gas impermeable plastic film, and a plastic cap was placed over the tube to reduce the exchange of air and trap the volatiles released by heating the cabbage-amended soil.

The test tubes were placed in the water baths at 25°C until the temperature of the soil and the water were equilibrated, and then the cycling was initiated. Temperatures were monitored using a CR10 datalogger (Campbell Scientific, Inc., Logan, Utah 84321). Three tubes were removed after 1, 2, and 3 days, and every 3 days thereafter during a period of 24 days. Part of the soil (15 g) was diluted with soft agar and plated on PARP within 24 hours, as previously described. The number of colonies was recorded. The other part of the soil was used in a plant disease assay.

At the end of the thermocycling experiment, all samples were transferred to 50-ml, tripour plastic beakers and one, 1-month-old tomato seedling was transplanted into the soil in each beaker. A small amount of vermiculite was poured on the top of the soil to prevent the roots from desiccating. All plants were kept in an growth chamber at 27°C for 30 days. As plants died, the root systems were rinsed in water, surface disinfested for 30 seconds in 70% ethanol, rinsed twice in sterile water, and plated on PARP. At the end of the experiment, all plants were cut and the root systems plated on PARP for determination of infection.

The use of average temperatures achieved during soil solarization for the temperature cycling experiments was not sufficient to completely inactivate chlamydospores of *P. nicotianae*; therefore, an additional experiment was conducted using temperature regimes achieved during an optimum solarization day at the NFREC-Quincy in 1995. Temperature regimes used were 47°C for 3 hours, 44°C for 5 hours, and35°C for 8 hours daily. Throughout this experiment a baseline temperature of 25°C was maintained during the rest of the day. The duration of each experiment was 15 days, and the experiment was repeated once.

The procedures for production of chlamydospores, treatment of soil, and estimation of soil populations of *P. nicotianae* were the same as described previously. At

the end of the temperature cycling experiments, a plant disease assay was performed for 4 weeks with 1-month-old tomato seedlings, as described previously. All dead plants were plated on PARP to confirm the presence of mycelium typical of *Phytophthora* spp., and at the end of the experiment all surviving plants were plated to determine if any infection had occurred.

The Effect of Soil Water Matric Potential, Temperature Regimes, and Cabbage Amendments on the Thermal Inactivation of Chlamydospores of *Phytophthora nicotianae*

The effect the soil water matric potential in association with temperature regimes that simulate soil solarization and cabbage amendments was examined. The soil water matric potentials used were 0 kPa, -10 kPa and -30 kPa. These potentials were selected due to their importance on the life cycle of the pathogen. The temperature regimes used were 1.5 hours at 44°C, 5 hours at 41°C and 8 hours at 35°C, or 3 hours at 47°C, 5 hours at 44°C and 8 hours at 35°C, with a baseline temperature of 25°C during the rest of the day. These temperatures were maintained with circulation heaters in water baths. The experiment was repeated once.

The procedures for production of chlamydospores, treatment of soil, and estimation of surviving populations of *P. nicotianae* were the same as described previously. At the end of the temperature cycling experiments, a plant disease assay was performed for 4 weeks with 1-month-old tomato seedlings, as described previously. All dead plants were plated on PARP to confirm the presence of mycelium typical of *Phytophthora* spp., and at the end of the experiment all surviving plants were plated to determine if any infection had occurred.

The Effect of Three Nonpasteurized Soils, Temperature Regimes and Cabbage Amendments on the Thermal Inactivation of Chlamydospores of *Phytophthora nicotianae*

The effects of nonpasteurized soils and cabbage amendments were examined using soil from the three field sites where soil solarization was evaluated from 1994 to 1996 (see Chapter 2 for a description of the soil types). The soil water matric potential of all soils was adjusted to -10 kPa, and the following temperature regimes were evaluated in one test, with three replicates per combination of soil, pasteurization, temperature and exposure time: 47°C for 3 hours, 44°C for 5 hours, 44°C for 1.5 hours, 41°C for 5 hours, and 35°C for 8 hours. The experiment was repeated once.

The procedures for production of chlamydospores, treatment of soil, and estimation of surviving populations of *P. nicotianae* were the same as described previously. At the end of the temperature cycling experiments, a plant disease assay was performed for 4 weeks with 1-month-old tomato seedlings, as described before. This assay was done with the soil from temperature regimes of 41°C and above, since all previous experiments indicated that all of the plants were infected at the lower temperature regimes after 30 days. All dead plants were plated on PARP, amended with 50 mg of hymexazol per liter of medium to confirm the presence of mycelium typical of *Phytophthora* spp., and at the end of the experiment all surviving plants were plated to determine if infection had occurred.

Statistical Analysis

The results of each experiment were analyzed individually. Whenever statistical analysis of the residues indicated that the results could be pooled due to the lack of variation, a final analysis was done with the pooled data.

The response surfaces were analyzed using the procedure PROC NLIN of SAS (SAS Institute, Cary, NC; release 6.11 for personal computers). Survival data were

transformed using ln (ppg+1) prior to analysis. Comparisons between the qualitative and quantitative methods used to determine the number of chlamydospores surviving the heat treatment were made.

The analyses of the effect of temperature cycling and cabbage amendment on the survival of chlamydospores of *P. nicotianae* were performed with Statgraphics Plus, version 2.1 (Manugistics, Inc., Rockville, MD), for the convenience of comparing several linearization models at once.

The experiments on the determination of the effects of soil water matric potential and the effects of different soils on survival of *P. nicotianae* were analyzed using PROC GLM of SAS for the analysis of variance. The determination of the effect of each individual factor was calculated as the average of that factor across all other factors; the analysis of each interaction was done by calculating the average for the secondary factor within the main factor across all other factors.

Results

Effect of Constant Temperature on the Inactivation of Chlamydospores of *Phytophthora nicotianae*

Phytophthora nicotianae was consistently recovered throughout the 2-hour experiment when temperatures were below 47°C (Table 3.1). At 44°C, a sharp decrease was observed after 30 minutes, but 10% of the initial population (500 ppg) survived to the end of the experiment. After 75 minutes of heat treatment at 47°C, population levels dropped below 1 propagule per gram of soil, and after 2 hours populations had declined to levels undetectable by the procedure used to quantify the inoculum in the soil. At 50° and 53°C, populations were very low after only 5 minutes of exposure.

Table 3.1. Effect of constant temperature and time on the survival of chlamydospores of *Phytophthora nicotianae*.

Temperature	Number of propagules per gram of soil recovered over time (minutes)[x]									
(°C)	5	10	15	30	45	60	75	90	105	120
25	110.5[y]	133.2	140.8	150.7	149.7	142.1	165.5	176.7	179.0	323.4
35	184.2	190.9	182.3	152.9	144.0	149.4	134.0	125.4	159.4	153.0
38	64.6	93.7	82.8	91.0	80.5	114.3	99.8	109.9	137.3	107.6
41	178.5	152.2	161.7	193.7	220.7	210.3	87.6	88.1	92.7	88.8
44	108.0	118.0	112.8	60.4	50.4	40.2	35.8	40.5	46.9	49.2
47	18.5	12.2	10.3	3.5	1.4	1.3	0.7	0.5	0.4	0.0
50	2.6	1.6	0.7	0.4	0.2	-	-	-	-	-
53	0.3	0.1	0.1	-[z]	-	-	-	-	-	-

[x] Soil initially infested with 500 chlamydospores per gram; *P. nicotianae* recovered on a selective medium.

[y] Each point consists of the average of two experiments with three repetitions each.

[z] Not tested.

The decline in survival of the chlamydospores of *P. nicotianae* in soil exposed to constant heat above 38°C for 5 to 120 minutes was best described by the equation $ln(\text{ppg}+1) = (6.2 - 0.00108 * \text{time}^2) * e^{-0.02208 * \text{temperature}}$, based on the comparison of the plotting of Observed , Predicted Values, Residuals X Time, and Residuals X Temperature, and on the $R^2_{adj} = 0.8234$, in which ppg is the number of propagules recovered per gram of soil.

The use of tomato seedlings as bait for the detection of survival of *P. nicotianae* indicated that survival was affected by the heat treatment only after 90% of the initial population of chlamydospores had been inactivated, which occurred generally only after exposure to 47°C or higher (Tables 3.1 and 3.2). The detection of survival with the use of seedlings was not as sensitive as the detection with the soil dilution procedure used. For example, at 53°C no seedlings were infected after the 3-day incubation period (Table 3.2); in contrast, the soil dilution procedure indicated that 0.1 propagule per gram of soil were still alive after 15 minutes of treatment (Table 3.1).

Exposure of chlamydospores of *P. nicotianae* to heat for 480 hours indicated that the pathogen can survive in the at 35°C for a long period (Table 3.3). Populations declined to levels below 1 propagule per gram of soil when temperatures were maintained at 38°C for more than 288 hours. After 96 hours of exposure at 41°C, or 48 hours at 44°C, no propagules were recovered from the soil. Exposure to 47°C reduced the populations to residual levels after 4 hours.

The decline in survival of the chlamydospores of *P. nicotianae* in soil exposed to constant temperatures above 38°C for 2 to 480 hours was best described by the equation $ln(\text{ppg}+1) = 6.2 * e^{(-0.00759 * \text{time} - 0.0138 * \text{temperature})}$, based on the comparison of the plotting of Observed X Predicted Values, Residuals X Time, and Residuals X Temperature, and on the $R^2_{adj} = 0.6773$, in which ppg is the number of propagules recovered per gram of soil.

Table 3.2. Detection of *Phytophthora nicotianae* by the tomato seedling assay after heat treatment for 5 to 120 minutes.

Temperature (°C)	Percentage of colonized tomato seedlings after soil treatment over time (minutes)									
	5	10	15	30	45	60	75	90	105	120
25	100[y]	100	100	100	100	100	100	100	100	100
35	100	100	100	100	100	100	100	100	100	100
38	100	100	100	100	100	100	100	100	100	100
41	100	100	100	100	100	100	100	100	100	100
44	100	100	100	100	100	100	100	100	100	100
47	78	50	94	72	61	44	22	11	17	33
50	17	17	0	17	0	-	-	-	-	-
53	0	0	0	-[z]	-	-	-	-	-	-

[y] Each point consists of the average of two experiments with three repetitions each.
[z] Not tested.

Table 3.3. Effect of constant temperature and extended periods of time on the survival of chlamydospores of *Phytophthora nicotianae*.

Temperature	Number of propagules per gram of soil recovered over time (hours)[x]												
(°C)	2	4	8	12	16	20	24	48	96	192	288	384	480
25	300.9[y]	214.6	270.1	271.8	274.9	290.7	329.4	524.2	326.5	294.1	275.7	297.2	241.8
35	150.9	231.5	344.8	430.0	362.6	398.5	402.8	385.1	461.2	447.4	311.5	88.1	68.9
38	125.8	162.8	268.3	300.1	354.0	397.2	417.1	89.8	34.9	9.5	0.9	0.2	0.0
41	146.0	169.6	150.6	91.6	99.1	78.3	50.4	11.7	0.0	0.0	0.0	0.0	0.0
44	35.2	10.2	1.6	0.1	0.0	0.1	0.1	0.0	0.0	-	-	-	-
47	0.7	0.1	-[z]	-	-	-	-	-	-	-	-	-	-

[x] Soil initially infested with 500 chlamydospores per gram; *P. nicotianae* recovered on a selective medium.

[y] Each point consists of the average of three experiments with three repetitions each.

[z] Not tested.

Generally, the use of the tomato seedling assay provided similar results to the soil dilution plating procedure, except when only residual inoculum (0.1 propagule per gram of soil) was left (Tables 3.3, 3.4). When residual inoculum survived the heat treatment, baiting the soil with seedlings allowed detection of the fungus in two cases that were not detected by soil plating, but failed to detect the pathogen in three other instances that were detected by the plating procedure. All tomato seedlings were colonized by *P. nicotianae* whenever more than 50 propagules per gram of soil were recovered in the soil dilution plates with PARP; in one case 100% infection was observed when 10 propagules per gram of soil were recovered.

The Effect of Cabbage Amendments on the Thermal Inactivation of Chlamydospores of *Phytophthora nicotianae*

The addition of dried, ground cabbage leaves changed the response of the chlamydospores to heat (Table 3.5). As concentration of cabbage increased, inactivation also increased; thus, less time was required to inactivate spores in cabbage amended soil.

At 35°C chlamydospores survived in the soil for 480 hours when no cabbage amendment was present (Table 3.5). The addition of 0.125% cabbage to the soil reduced the population to residual levels after 192 hours. Ninety six hours were required with 0.25% cabbage to reduce population counts below one propagule per gram of soil; similar population reductions were attained after 48 hours when 0.5% cabbage was added to the soil. The addition of cabbage to soils incubated at 38°C reduced pathogen populations in the following manner: the time required to reduce the inoculum to below 1 propagule per gram of soil dropped from 192 hours without cabbage amendment to 48 hours with 0.125% or 0.25% amendment, and to 24 hours with 0.5% amendment added to the soil. Survival of the chlamydospores in soil treated at 41°C declined to undetectable levels

Table 3.4. Detection of *Phytophthora nicotianae* by the tomato seedling assay after heat treatment for 2 to 480 hours.

Temperature	Percentage of colonized tomato seedlings after soil treatment over time (hours)												
(°C)	2	4	8	12	16	20	24	48	96	192	288	384	480
25	100[y]	100	100	100	100	100	100	100	100	100	100	100	100
35	100	100	100	100	100	100	100	100	100	100	100	100	100
38	100	100	100	100	100	100	100	100	96	77	52	37	0
41	100	100	100	100	100	100	95	41	18	0	0	0	0
44	89	100	42	0	4	0	0	0	0	-	-	-	-
47	52	26	-[z]	-	-	-	-	-	-	-	-	-	-

[y] Each point consists of the average of three experiments with three repetitions each.
[z] Not tested.

Table 3.5. Effect of constant temperature, cabbage amendment, and time on the inactivation of chlamydospores of *Phytophthora nicotianae*.

Temperature	Cabbage	Number of propagules per gram of soil recovered over time (hours)[x]												
(°C)	(%)	2	4	8	12	16	20	24	48	96	192	288	384	480
35	0	400.7[y]	415.1	327.8	434.6	472.6	471.4	379.9	149.2	226.0	159.2	68.2	0.0	26.5
35	0.125	102.8	77.0	79.6	79.8	60.9	58.3	36.8	35.2	1.5	0.1	0.0	0.0	0.0
35	0.25	116.5	57.7	102.2	38.0	42.6	44.7	24.7	2.8	0.6	0.0	0.0	0.0	0.1
35	0.5	101.0	96.1	53.8	39.2	18.1	17.1	13.1	0.5	0.0	0.0	0.0	0.0	0.0
38	0	202.0	185.9	146.5	280.7	182.1	201.7	218.6	41.9	3.6	0.1	0.0	0.0	0.5
38	0.125	88.5	91.2	82.8	67.2	80.6	15.2	4.9	0.0	0.0	0.0	0.0	0.0	0.0
38	0.25	100.6	73.2	57.3	48.0	27.2	8.2	8.5	0.1	0.0	0.0	0.0	0.0	0.0
38	0.5	94.8	76.1	32.8	15.2	7.4	2.7	0.7	0.0	0.0	0.0	0.0	0.0	0.0
41	0	309.1	236.4	210.6	203.3	106.5	15.9	14.8	4.6	0.0	-[z]	-	-	-
41	0.125	99.8	79.5	30.6	16.6	10.4	2.9	0.6	0.0	0.0	-	-	-	-
41	0.25	72.7	40.4	9.9	4.0	2.0	1.9	0.0	0.0	0.0	-	-	-	-
41	0.5	67.9	43.5	6.6	1.3	0.2	0.0	0.0	0.0	0.0	-	-	-	-

[x] Soil initially infested with 500 chlamydospores per gram.

[y] Each point consists of the average of two experiments with three repetitions each.

[z] Not tested.

after 96 hours in nonamended soil, 48 hours in soil amended with 0.125% cabbage, and 24 hours in soil amended with 0.25% cabbage (Table 3.5). Only 16 hours were required to reduce the population to 0.2 propagule per gram of soil in soil amended with 0.5% cabbage.

The decline in survival of the chlamydospores of *P. nicotianae* in soil amended with cabbage residue and exposed to constant temperature was best described by the linear-power model presented in Table 3.6. According to the model used for comparisons of survival of *P. nicotianae*, the equation describing the inactivation of chlamydospores in the absence of cabbage amendment is significantly different from the others ($P \leq 0.05$); the equations generated for amendment with 0.125% and 0.25% cabbage are not different from each other, and the equation generated for amendment with 0.5% cabbage is different from all other equations.

Detection of survival of *P. nicotianae* with tomato seedlings as baits followed similar trends as those observed with the soil dilution plating procedure (Tables 3.5, 3.7). As the number of propagules recovered in the soil plates decreased from 500 to 100 propagules per gram of soil, the percentage of colonized seedlings was relatively constant. Further decreases in the propagule counts generally reflected a decrease in the percentage of colonized seedlings. Of the 41 cases in which no propagules were detected by the soil dilution plating procedure, the survival of the pathogen was detected in 17 cases by the baiting technique. Generally, this discrepancy was observed right after the number of propagules recovered in the selective medium had dropped below 1 propagule per gram of soil.

Table 3.6. Equations of models that describe the thermal inactivation of chlamydospores of *Phytophthora nicotianae* as a factor of constant temperature between 35°C and 41°C, time of exposure ranging from 2 to 480 hours, and cabbage amendment concentrations.

Cabbage (%)	Equation	$R^2_{adj.}$
0	$ln(ppg^z + 1) = (6.2 - 0.0141 * \text{Time}) * e^{-0.0063 * \text{Temperature}}$	0.5062
0.125	$ln(ppg + 1) = (6.2 - 0.0181 * \text{Time}) * e^{-0.0191 * \text{Temperature}}$	0.5474
0.25	$ln(ppg + 1) = (6.2 - 0.0183 * \text{Time}) * e^{-0.0232 * \text{Temperature}}$	0.6124
0.5	$ln(ppg + 1) = (6.2 - 0.0187 * \text{Time}) * e^{-0.0282 * \text{Temperature}}$	0.6928

[z] ppg = propagules per gram of soil.

Table 3.7. Detection of *Phytophthora nicotianae* by the tomato seedling assay after heat treatment of cabbage amended soil for 2 to 480 hours.

Temperature	Cabbage	Percentage of colonized tomato seedlings after soil treatment over time (hours)												
(°C)	(%)	2	4	8	12	16	20	24	48	96	192	288	384	480
35	0	100[y]	100	100	100	100	100	100	100	100	100	83	11	33
35	0.125	100	100	100	100	100	100	100	100	83	17	0	0	22
35	0.25	100	100	100	100	100	100	100	100	17	6	6	0	22
35	0.5	100	100	100	100	89	100	100	89	33	0	0	0	0
38	0	100	100	100	100	100	100	100	100	67	17	0	0	6
38	0.125	83	56	100	100	56	10	100	11	33	0	0	0	0
38	0.25	100	78	100	100	72	94	83	6	28	0	0	0	0
38	0.5	100	100	100	100	50	72	56	11	28	11	0	0	0
41	0	100	100	100	100	100	100	100	100	44	-[z]	-	-	-
41	0.125	89	50	50	61	39	50	50	0	22	-	-	-	-
41	0.25	94	56	50	33	28	33	0	11	22	-	-	-	-
41	0.5	100	83	67	17	28	6	0	0	11	-	-	-	-

[y] Each point consists of the average of two experiments with three repetitions each.
[z] Not tested.

The Effect of Cycling Temperatures and Cabbage Amendments on the Thermal Inactivation of Chlamydospores of *Phytophthora nicotianae*

Generally, the use of pulsing temperatures increased the time required to inactivate the chlamydospores of *P. nicotianae* in comparison with the constant temperature treatment (Tables 3.5, 3.8). After 24 days at 35°C for 8 hours daily, 40% of the initial infestation level of spores (500 chlamydospores per gram of soil) was still recovered from the nonamended soil in the selective medium. The addition of 0.125% cabbage to the soil reduced the population to levels below 10% after 18 days. The effect of cabbage was evident from the very first cycles. Cycling temperatures at 41°C for 5 hours daily reduced the survival of the spores by more than 90% of the initial infestation level after 24 days; when cabbage was added, less than 5% of the propagules were recovered from the soil at the end of the experiment. Survival was below 5% of the initial infestation level after 21 days of exposure to 1.5 hours at 44°C daily; with cabbage amendment, detection was lower than that observed in the nonamended soil.

Exposure to temperature regimes simulating optimum solarization periods reduced the populations to very low levels (Table 3.8). Five hours daily at 44°C for 15 days reduced the population to 0.4 propagule per gram of soil. The use of cabbage amendment reduced populations to the same levels after 6 days. Only 3 days of exposure at 47° for 3 hours daily were required to reduce populations to levels below 1 propagule per gram of soil; with cabbage amendment populations were reduced to levels below 1 propagule per gram of soil after 2 days. Populations were reduced to levels below detection only at the two highest temperature regimes (44°C for 5 hours daily and 47°C for 3 hours daily) after 9 days of treatment. Soil amendment with cabbage appeared to have a greater impact at lower temperatures, where the heat alone was not sufficient to inactivate the chlamydospores.

Table 3.8. The effect of temperature regime, cabbage amendment, and time on the survival of chlamydospores of *Phytophthora nicotianae*.

Temperature	Cabbage	Number of propagules per gram of soil recovered over time (days)									
Regime[y]	(%)	1	2	3	6	9	12	15	18	21	24
35-8	0	321.9	336.4	304.1	334.6	342.2	314.1	324.4	256.7	234.0	199.3
35-8	0.125	114.5	121.2	129.4	130.5	108.8	84.0	80.8	41.7	32.9	30.9
41-5	0	251.4	329.1	359.0	353.0	250.2	247.2	155.9	80.7	51.9	41.3
41-5	0.125	47.8	58.2	42.6	41.3	37.2	22.2	24.5	18.6	21.5	18.7
44-1.5	0	98.0	93.8	91.5	75.5	41.0	36.8	33.2	25.3	22.0	19.8
44-1.5	0.125	31.4	33.1	35.0	24.0	20.0	16.8	15.6	16.3	12.6	14.4
44-5	0	47.2	34.0	26.0	5.2	2.5	1.7	0.4	-[z]	-	-
44-5	0.125	34.4	14.6	2.7	0.4	0.0	0.0	0.0	-	-	-
47-3	0	3.2	1.2	0.3	0.1	0.2	0.0	0.1	-	-	-
47-3	0.125	2.2	0.1	0.0	0.1	0.0	0.0	0.0	-	-	-

[x] Soil initially infested with 500 chlamydospores per gram.

[y] Temperature regimes that simulated solarization consisted of temperatures increased daily to 35°, 41°, 44°, 44°, or 47°C for 8, 5, 1.5, 5 or 3 hours, respectively; the remainder of each day was maintained at 25°C.

[z] Not tested.

The reduction in survival of *P. nicotianae* was modeled using linear regression. The model selected for the comparisons among temperature regimes and cabbage amendment was $\ln(Y+1) = a + b*X$, in which Y is the number of propagules recovered per gram of soil and X is the number of days of exposure to the temperature regime. A lack of fit of the model to the data was observed at 35°C, regardless of the cabbage amendment (Table 3.9). As temperature increased a better fit of the model was observed, but at the highest temperature regime tested a lower fit was obtained again.

The plant disease assay verifying the pathogenicity of the surviving populations of *P. nicotianae* indicated that temperature regimes below 44°C for 5 hours daily did not reduce the infection of the tomato seedlings (Table 3.10). At 44°C for 5 hours, infection was reduced in the soil amended with cabbage after 3 days. After 3 days of heat treatment at 47°C for 3 hours daily, no infection of seedlings was observed. Mortality of the seedlings was observed in the lower temperature regimes (Table 3.11); however, when the soil was treated at 44°C for 5 hours daily no seedlings died, except after 2 or 3 days in soil amended with cabbage. No seedlings died when the soil was heated to 47°C for 3 hours daily.

The Effect of Soil Water Matric Potential, Temperature Regimes, and Cabbage Amendments on the Thermal Inactivation of Chlamydospores of *Phytophthora nicotianae*

The temperature regimes evaluated in these tests simulated either average daily periods or optimum daily temperature periods observed during solarization. Generally, as temperature within the regimes increased, the number of chlamydospores recovered in the soil dilution plates decreased (Tables 3.12, 3.13, 3.14). Propagule survival in the two temperature regimes simulating optimum solarization periods (47°C for 3 hours and 44°C

Table 3.9. Regression coefficients of the linear regressions of temperature regimes and survival of chlamydospores of *Phytophthora nicotianae*; the model used for the analysis was ln (ppg+1) = a + b*days.

Temperature Regime[x]	Cabbage %	Parameters		R^2	P-Value[z]
		slope	intercept		
35-8	0	-0.01203	5.717	0.0267	0.062
35-8	0.125	-0.05508	4.808	0.2800	0.001
41-5	0	-0.09034	6.049	0.6679	0.001
41-5	0.125	-0.05091	3.812	0.2195	0.001
44-1.5	0	-0.07680	4.631	0.7264	0.001
44-1.5	0.125	-0.04426	3.454	0.2856	0.001
44-5	0	-0.26132	3.886	0.8684	0.001
44-5	0.125	-0.21618	2.531	0.5631	0.001
47-3	0	-0.05692	0.718	0.2422	0.001
47-3	0.125	-0.04026	0.455	0.2272	0.001

[x] Temperature regimes that simulated solarization consisted of temperatures increased daily to 35°, 41°, 44°, 44°, or 47°C for 8, 5, 1.5, 5 or 3 hours, respectively; the remainder of each day was maintained at 25°C.

[z] Significance associated to the coefficient of determination (R^2).

Table 3.10. Infection of tomato seedlings after incubation in soil amended with cabbage, infested with *Phytophthora nicotianae*, and heat treated over time.

Temperature	Cabbage	Percent infection of tomato seedlings over time (days)									
Regime[x]	(%)	1	2	3	6	9	12	15	18	21	24
35-8	0	100[y]	100	100	100	100	100	100	100	100	100
35-8	0.125	100	100	100	100	100	100	100	100	100	100
41-5	0	100	100	100	100	100	100	100	100	100	100
41-5	0.125	100	100	100	100	100	100	100	100	100	100
44-1.5	0	100	100	100	100	100	100	100	100	100	100
44-1.5	0.125	100	100	100	100	100	100	100	100	100	100
44-5	0	100	100	100	100	100	100	83	-[z]	-	-
44-5	0.125	100	100	67	67	50	67	33	-	-	-
47-3	0	100	100	0	0	0	0	0	-	-	-
47-3	0.125	100	83	0	0	0	0	0	-	-	-

[x] Temperature regimes that simulated solarization consisted of temperatures increased daily to 35°, 41°, 44°, 44°, or 47°C for 8, 5, 1.5, 5 or 3 hours, respectively; the remainder of each day was maintained at 25°C.

[y] Each data point consists of the average of two experiments with three repetitions each.

[z] Not tested.

Table 3.11. Mortality of tomato seedlings after incubation in soil amended with cabbage, infested with *Phytophthora nicotianae*, and heat treated over time.

Temperature	Cabbage	Percent mortality of tomato seedlings over time (days)									
Regime[x]	(%)	1	2	3	6	9	12	15	18	21	24
35-8	0	33[y]	27	27	27	33	33	33	33	44	44
35-8	0.125	33	27	20	40	27	40	33	44	44	33
41-5	0	57	33	33	33	33	44	33	33	44	56
41-5	0.125	44	33	44	33	44	33	33	44	44	22
44-1.5	0	44	44	33	44	33	22	33	11	22	22
44-1.5	0.125	33	33	33	33	33	45	0	22	11	11
44-5	0	0	0	0	0	0	0	0	-[z]	-	-
44-5	0.125	0	16	33	0	0	0	0	-	-	-
47-3	0	0	0	0	0	0	0	0	-	-	-
47-3	0.125	0	0	0	0	0	0	0	-	-	-

[x] Temperature regimes that simulated solarization consisted of temperatures increased daily to 35°, 41°, 44°, 44°, or 47°C for 8, 5, 1.5, 5 or 3 hours, respectively; the remainder of each day was maintained at 25°C.

[y] Each data point consists of the average of two experiments with three repetitions each.

[z] Not tested.

Table 3.12. The effect of temperature regimes that simulate daily solarization periods and cabbage amendment, at a soil water matric potential of 0 kPa, on the survival of chlamydospores of *Phytophthora nicotianae*, and on the percentages of infection and mortality of tomato seedlings after 30 days of exposure in the previously treated soil.

Temperature Regime[u]	Cabbage (%)	Days[v]	Test 1 PPG[w]	Test 1 I[x] (%)	Test 1 M[y] (%)	Test 2 PPG	Test 2 I (%)	Test 2 M (%)
35-8	0	1	223.08 e[z]	100	0	308.82 e	100	33
35-8	0	2	433.85 f	100	0	540.86 e	100	33
35-8	0	3	413.47 f	100	0	409.76 e	100	33
35-8	0	6	672.84 f	100	0	213.86 de	100	100
35-8	0.125	1	236.46 e	100	0	91.48 cde	100	33
35-8	0.125	2	226.47 ef	100	0	226.47 de	100	0
35-8	0.125	3	169.72 e	100	0	110.16 cde	100	17
35-8	0.125	6	153.47 e	100	0	67.58 cde	100	100
41-5	0	1	150.41 e	100	0	214.51 de	100	33
41-5	0	3	248.39 ef	100	0	104.64 cde	100	33
41-5	0	6	126.74 e	100	0	62.37 bcd	100	67
41-5	0.125	1	90.84 de	100	0	37.78 bcd	100	0
41-5	0.125	3	62.43 de	100	0	11.06 b	100	100
41-5	0.125	6	34.52 c	100	0	27.73 bc	100	33
44-1.5	0	1	34.16 cd	100	0	29.14 bc	100	33
44-1.5	0	3	19.29 bcd	100	0	15.61 b	100	67
44-1.5	0	6	14.80 bcd	100	0	10.32 b	100	67
44-1.5	0.125	1	49.91 de	100	0	42.90 bcd	100	33
44-1.5	0.125	3	54.15 de	100	0	12.46 b	100	67
44-1.5	0.125	6	20.98 cd	100	33	15.01 b	100	67
44-5	0	1	14.80 bcd	100	0	18.39 bc	100	0
44-5	0	2	7.76 bcd	100	0	11.26 b	100	0
44-5	0	3	6.54 bcd	100	0	9.32 b	100	0
44-5	0.125	1	6.17 bc	100	0	10.94 b	100	0
44-5	0.125	2	5.42 bc	100	0	6.61 b	100	0
44-5	0.125	3	3.62 bc	100	0	6.24 b	100	0
47-3	0	1	0.62 a	100	0	0.78 a	100	0
47-3	0	2	0.00 a	67	0	0.00 a	100	0
47-3	0	3	0.00 a	67	0	0.00 a	100	0
47-3	0.125	1	0.52 a	100	0	0.92 a	100	0
47-3	0.125	2	0.00 a	33	0	0.00 a	100	0
47-3	0.125	3	0.00 a	50	0	0.00 a	100	0

[u] Temperature regimes that simulated daily solarization periods consisted of temperatures increased daily to 35°, 41°, 44°C, 44°C or 47°C for 8, 5, 1.5, 5 or 3 hours, respectively; the temperature for the remainder of each day was maintained at 25°C.

[v] Days = duration of temperature regime.

[w] PPG = propagules per gram of soil; initial population was 500 chlamydospores per gram of soil.

[x] I = infection.

[y] M = mortality.

[z] Main effect means followed by the same letter in each test do not differ according to Tukey's Honestly Significant Difference procedure ($P \leq 0.05$); data were transformed to ln(ppg+1) for analysis and presented as weighted means ([Exp {mean}]-1).

Table 3.13. The effect of temperature regimes that simulate daily solarization periods and cabbage amendment, at a soil water matric potential of -10 kPa, on the survival of chlamydospores of *Phytophthora nicotianae*, and on the percentages of infection and mortality of tomato seedlings after 30 days of exposure in the previously treated soils.

Temperature Regime[u]	Cabbage (%)	Days[v]	Test 1 PPG[w]	I[x] (%)	M[y] (%)	Test 2 PPG	I (%)	M (%)
35-8	0	1	181.18 e	100	17	202.16 cd	100	0
35-8	0	2	247.14 e	100	0	287.59 d	100	0
35-8	0	3	168.02 e	100	0	147.41 cd	100	17
35-8	0	6	191.48 e	100	0	115.63 cd	100	67
35-8	0.125	1	325.69 e	100	0	218.42 d	100	50
35-8	0.125	2	274.06 e	100	67	274.06 d	100	0
35-8	0.125	3	200.34 e	100	0	115.51 cd	100	17
35-8	0.125	6	107.85 de	100	0	65.02 cd	100	33
41-5	0	1	449.34 e	100	0	258.82 d	100	33
41-5	0	3	261.43 e	100	0	109.50 cd	100	100
41-5	0	6	209.61 e	100	0	73.22 cd	100	67
41-5	0.125	1	215.16 e	100	0	146.67 cd	100	33
41-5	0.125	3	127.77 e	100	0	77.89 cd	100	67
41-5	0.125	6	97.20 de	100	0	55.15 cd	100	67
44-1.5	0	1	44.15 cd	100	0	14.26 b	100	33
44-1.5	0	3	19.70 cd	100	0	6.24 b	100	33
44-1.5	0	6	14.03 c	100	0	8.73 b	100	33
44-1.5	0.125	1	70.95 de	100	0	74.19 cd	100	0
44-1.5	0.125	3	59.95 d	100	0	78.44 cd	100	33
44-1.5	0.125	6	45.34 cd	100	33	39.77 cd	100	33
44-5	0	1	16.10 c	100	33	23.83 b	100	0
44-5	0	2	10.10 bc	100	0	16.99 b	100	0
44-5	0	3	4.64 b	100	0	6.92 b	100	0
44-5	0.125	1	8.38 bc	100	0	16.37 b	100	0
44-5	0.125	2	3.65 b	100	0	12.38 b	100	0
44-5	0.125	3	1.80 ab	100	0	8.48 b	100	0
47-3	0	1	2.24 b	100	0	3.13 ab	100	0
47-3	0	2	0.30 a	100	0	0.25 a	100	0
47-3	0	3	0.09 a	100	0	0.00 a	100	0
47-3	0.125	1	1.70 ab	100	0	5.75 b	100	0
47-3	0.125	2	0.52 a	100	33	0.45 a	100	0
47-3	0.125	3	0.45 a	100	0	0.28 a	100	0

[u] Temperature regimes that simulated daily solarization periods consisted of temperatures increased daily to 35°, 41°, 44°C, 44°C or 47°C for 8, 5, 1.5, 5 or 3 hours, respectively; the temperature for the remainder of each day was maintained at 25°C.

[v] Days = duration of temperature regime.

[w] PPG = propagules per gram of soil; initial population was 500 chlamydospores per gram of soil.

[x] I = infection.

[y] M = mortality.

[z] Main effect means followed by the same letter in each test do not differ according to Tukey's Honestly Significant Difference procedure ($P \leq 0.05$); data were transformed to ln(ppg+1) for analysis and presented as weighted means ([Exp {mean}]-1).

Table 3.14. The effect of temperature regimes that simulate daily solarization periods and cabbage amendment, at a soil water matric potential of -30 kPa, on the survival of chlamydospores of *Phytophthora nicotianae*, and on the percentages of infection and mortality of tomato seedlings after 30 days of exposure in the previously treated soils.

Temperature Regime[u]	Cabbage (%)	Days[v]	Test 1			Test 2		
			PPG[w]	I[x] (%)	M[y] (%)	PPG	I (%)	M (%)
35-8	0	1	225.11 e	100	50	90.81 d	100	33
35-8	0	2	190.71 e	100	0	221.96 d	100	0
35-8	0	3	185.98 e	100	17	161.71 d	100	33
35-8	0	6	207.30 e	100	33	114.93 d	100	0
35-8	0.125	1	349.37 e	100	0	137.80 d	100	17
35-8	0.125	2	385.45 e	100	0	385.45 d	100	0
35-8	0.125	3	230.60 e	100	17	107.96 d	100	50
35-8	0.125	6	133.29 de	100	0	63.07 cd	100	100
41-5	0	1	183.93 e	100	0	288.17 d	100	33
41-5	0	3	253.68 e	100	0	153.93 d	100	33
41-5	0	6	240.29 e	100	0	82.18 cd	100	33
41-5	0.125	1	371.78 e	100	0	116.20 d	100	67
41-5	0.125	3	124.21 de	100	0	80.29 cd	100	67
41-5	0.125	6	85.57 de	100	0	56.34 cd	100	33
44-1.5	0	1	65.75 d	100	0	25.21 c	100	33
44-1.5	0	3	19.41 c	100	0	6.43 b	100	0
44-1.5	0	6	14.77 bc	100	0	6.55 b	100	33
44-1.5	0.125	1	86.01 de	100	0	95.06 d	100	0
44-1.5	0.125	3	53.27 cd	100	0	30.88 c	100	67
44-1.5	0.125	6	42.73 cd	100	33	28.11 c	100	100
44-5	0	1	10.67 bc	100	0	9.86 bc	100	0
44-5	0	2	9.52 bc	100	0	7.35 bc	100	0
44-5	0	3	5.83 b	100	33	4.80 b	100	0
44-5	0.125	1	9.31 bc	100	0	14.25 bc	100	0
44-5	0.125	2	6.01 b	100	0	8.55 bc	100	0
44-5	0.125	3	3.23 b	100	0	7.66 bc	100	0
47-3	0	1	1.90 ab	100	0	1.44 ab	100	0
47-3	0	2	0.09 a	100	0	0.10 a	100	0
47-3	0	3	0.09 a	100	0	0.28 a	100	0
47-3	0.125	1	2.79 b	100	0	4.07 b	100	0
47-3	0.125	2	0.79 a	100	0	1.15 ab	100	0
47-3	0.125	3	0.91 a	100	0	0.86 ab	100	0

[u] Temperature regimes that simulated daily solarization periods consisted of temperatures increased daily to 35°, 41°, 44°C, 44°C or 47°C for 8, 5, 1.5, 5 or 3 hours, respectively; the temperature for the remainder of each day was maintained at 25°C.

[v] Days = duration of temperature regime.

[w] PPG = propagules per gram of soil; initial population was 500 chlamydospores per gram of soil.

[x] I = infection.

[y] M = mortality.

[z] Main effect means followed by the same letter in each test do not differ according to Tukey's Honestly Significant Difference procedure (P≤ 0.05); data were transformed to ln(ppg+1) for analysis and presented as weighted means ([Exp {mean}]-1).

for 5 hours daily) were significantly lower than those in the base temperature regime of 35°C for 8 hours daily, and generally lower than those treated at 41°C for 5 hours daily, regardless of the soil water matric potential ($P \leq 0.05$).

The use of a plant disease assay to confirm the pathogenicity of the surviving population of *P. nicotianae* indicated that, when the soil was maintained at 0 kPa and treated at 47°C for 3 hours daily, there was a reduction in infection of the seedlings in test 1 only (Table 3.12). In all other treatments under all three matric potentials, all of the seedlings were infected by *P. nicotianae* (Tables 3.12, 3.13, 3.14). A higher proportion of mortality of seedlings was observed in the second experiment than in the first experiment; however, little or no mortality occurred at any soil water matric potential when the soil was treated at 44°C for 5 hours daily or at 47°C for 3 hours daily.

Over all treatments, in both tests, soil water matric potential had a significant effect in reducing the populations of *P. nicotianae* in the soil ($P \leq 0.05$) (Table 3.15; Appendix D). In both tests the lowest survival was observed in saturated soils (0 kPa) (Tables 3.12, 3.13, 3.14; Appendix D). No differences were observed in the survival of chlamydospores in soils maintained at either -10 kPa or -30 kPa in test 1; however, in test 2 survival at -10 kPa was higher than at -30 kPa.

As temperature increased, survival of *P. nicotianae* decreased (Table 3.15; Appendix D), and survival at each temperature regime was significantly different from that in all others ($P \leq 0.05$). Survival was lowest in soil maintained at 47°C for 3 hours daily (Appendix D).

The incorporation of cabbage amendment into soil significantly reduced the populations of *P. nicotianae* in test 1, while no effect on the survival of the pathogen was observed in test 2 ($P \leq 0.05$) (Table 3.15, Appendix D). However, differences in populations between amended and nonamended soil exposed to specific temperature regimes were not evident from the analysis (Tables 3.12, 3.13, 3.14).

Table 3.15. Analysis of variance of the effect of temperature regimes that simulate solarization periods, cabbage amendment, and soil water matric potential on the survival of chlamydospores of *Phytophthora nicotianae*

Source of Variation		Test 1			Test 2		
	df	MS	F	P	MS	F	P
Soil water matric potential (Ψ_m)[w]	2	1.355	15.93	0.0001	1.967	16.68	0.0001
Temperature[x]	4	264.897	3114.24	0.0001	210.227	1782.67	0.0001
Cabbage[y]	1	0.924	10.87	0.0001	0.001	0.01	0.9149
Time[z]	3	6.740	79.24	0.0001	11.725	99.43	0.0001
Ψ_m x Temperature	8	1.096	12.89	0.0001	1.366	11.59	0.0001
Ψ_m x Cabbage	2	1.827	21.48	0.0001	6.789	57.57	0.0001
Ψ_m x Time	6	0.375	4.41	0.0003	0.133	1.13	0.3442
Temperature x Cabbage	4	4.658	54.77	0.0001	8.255	70.01	0.0001
Temperature x Time	8	0.873	10.26	0.0001	2.024	17.16	0.0001
Cabbage x Time	3	0.643	7.57	0.0001	0.036	0.30	0.8230
Temperature x Ψ_m x Cabbage x Time	54	0.225	2.65	0.0001	0.265	2.25	0.0001
Residual	228	0.085			0.118		

[w] Soil water matric potential adjusted to 0, -10, or -30 kPa.

[x] Temperature regimes that simulated daily solarization periods consisted of temperatures increased daily to 35°, 41°, 44°C, 44°C or 47°C for 8, 5, 1.5, 5 or 3 hours, respectively; the temperature for the remainder of each day was maintained at 25°C.

[y] Soil amended or not amended with dry, ground cabbage at a rate of 0.125% (w/w).

[z] Time = number of days of exposure to heat treatment at a given temperature regime.

Generally, the longer the soil infested with chlamydospores of *P. nicotianae* was exposed to the heat treatments, the lower the survival of the pathogen (Tables 3.12, 3.13, 3.14, 3.15). However, the effect of time has to be analyzed with care because of the different durations of exposure to the temperature regimes. Because the time required to kill chlamydospores is inversely proportional to temperature, shorter sampling times (1, 2, and 3 days) were used at the higher temperature regimes (47°C for 3 hours daily and 44°C for 5 hours daily) and longer sampling times (1, 3, and 6 days) were used at the average temperature regimes (44°C for 1.5 hours daily and 41°C for 5 hours daily); the base temperature (35°C for 8 hours daily) was sampled at all time intervals (1, 2, 3, and 6 days). Therefore, the analysis of the effect of time across all other factors is biased in the following manner: the data for 2 days of exposure was obtained only from the temperature regimes that simulate optimum solarization periods and the base temperature regime; after 3 days of heat treatment, a final sample was collected from the optimum temperature regimes, and intermediate samples were collected from the average temperature regimes; after 6 days only the average temperature regimes and the base temperature regime were evaluated. Consequently, artificially higher survival rates were generated at 6 days as compared to 2 or 3 days because the lowest survival rates under the optimum temperature regimes had not been determined (Appendix D). The interactions of soil water matric potential or cabbage with time reflect the same bias, and the responses followed trends similar to those described here for time alone. Although these two interactions were significant (Table 3.15), the biased results did not allow insight into the roles of soil water matric potential or cabbage amendment in relation to time of exposure on the survival of the pathogen.

Within each of the soil water matric potentials evaluated, each of the temperature regimes significantly reduced the populations of *P. nicotianae* in relation to the base temperature (35°C for 8 hours daily) ($P \leq 0.05$) (Tables 3.12, 3.13, 3.14, 3.15; Appendix

D). Survival of *P. nicotianae* at each temperature regime was significantly lower than that at all other preceding temperature regimes.

Although there was a significant interaction of soil water matric potential and cabbage amendment, the effect of the amendment on survival of *P. nicotianae* was not consistent throughout the range of soil water matric potentials evaluated ($P \leq 0.05$) (Tables 3.12, 3.13, 3.14, 3.15; Appendix D). Cabbage amendment reduced the survival of propagules in soil maintained at 0 kPa (Appendix D). However, when the soil was maintained at -30 kPa, higher survival of the pathogen was observed in soil amended with cabbage than in nonamended soil. At -10 kPa, the addition of cabbage to the soil had no impact on the survival of *P. nicotianae* in test 1, while in test 2 survival was greater in soil amended with cabbage than in nonamended soil.

The effect of cabbage amendment on survival of *P. nicotianae* within each temperature regime was significant, but not consistent ($P \leq 0.05$) (Table 3.15; Appendix D). At the two lower temperature regimes (35°C for 8 hours and 41°C for 5 hours daily) the addition of cabbage to the soil reduced the number of propagules of *P. nicotianae* recovered on the selective medium (Tables 3.12, 3.13, 3.14). At 44°C for 1.5 hours, and 47°C for 3 hours daily, a higher proportion of the population of *P. nicotianae* survived in soil that was amended with cabbage than in nonamended soil. In test 1, at 44°C for 5 hours daily the number of propagules of the pathogen recovered was lower in the soil amended with cabbage than in nonamended soil; in contrast, in test 2 there were no differences in the survival of *P. nicotianae* in soils amended or nonamended with cabbage.

Generally, longer exposure of the infested soil to each temperature resulted in lower recovery of propagules (Tables 3.12, 3.13, 3.14, 3.15; Appendix D). At the base temperature regime of 35°C for 8 hours daily, the lowest survival was observed after 3 or 6 days. In test 1 no differences in survival were observed between 3 and 6 days or between 1 and 2 days of exposure to the heat treatment. In contrast, in test 2, the highest

survival was observed after 2 days of exposure, followed by 1 and 3 days of exposure to the heat treatment. When average temperature regimes were employed, the reduction in survival was not as consistent as with the optimum temperature regimes. At 41°C for 5 hours daily, and 44°C for 1.5 hours, survival decreased with increasing length of exposure in test 1; however, in test 2 no differences were observed between 3 and 6 days of exposure, in which survival was lower than after exposing the soil to 1 day of heat treatment. As time progressed, lower recovery was observed at 44°C for 5 hours daily. Exposing the chlamydospore-infested soil to either 2 or 3 days at 47°C for 3 hours daily resulted in lower survival as compared with a 1-day exposure.

The Effect of Three Nonpasteurized Soils, Temperature Regimes, and Cabbage Amendments on the Thermal Inactivation of Chlamydospores of *Phytophthora nicotianae*

Soils from the three sites where soil solarization was tested were used in this study. In all three soils the two higher temperature regimes (44°C for 5 hours and 47°C for 3 hours daily) were generally more effective in the inactivation of chlamydospores of *P. nicotianae* than the two lower regimes (35°C for 8 hours and 41°C for 5 hours daily) (Tables 3.16, 3.17, 3.18). Very few propagules were recovered from soil from Site 1 after 9 days at the temperature regime of 47°C for 3 hours daily, and no propagules were recovered from the other soils after this treatment.

Few tomato seedlings died after 30 days of incubation in the treated soils (Table 3.16, 3.17, 3.18). However, almost all seedlings were infected in all treatments, except at the highest temperature regime of 47°C for 3 hours daily. At the highest temperature regime, infection was observed in the second test, even in treatments where no propagules were recovered. In contrast, in the first test, no infection of the root system

Table 3.16. The effect of pasteurization of soil from Site 1 (NTG farm, Decatur County, Georgia), temperature regimes, and cabbage amendment on the survival of chlamydospores of *Phytophthora nicotianae*, and on the percentages of infection and mortality of tomato seedlings after 30 days of exposure in the previously treated soils.

			Test 1						Test 2					
Temperature			Pasteurized soil			Nonpasteurized soil			Pasteurized soil			Nonpasteurized soil		
Regime[t]	Cabbage (%)	Time[u] (days)	PPG[v]	I[w] (%)	M[x] (%)	PPG	I (%)	M (%)	PPG	I (%)	M (%)	PPG	I (%)	M (%)
35-8	0	3	15.6 bcd[y]	nt[z]	nt	34.6 cd	nt	nt	3.7 bc	nt	nt	109.8 d	nt	nt
35-8	0	9	29.4 cd	nt	nt	102.2 d	nt	nt	107.2 d	nt	nt	71.5 d	nt	nt
35-8	0.125	3	66.0 d	nt	nt	62.4 d	nt	nt	8.7 bc	nt	nt	139.6 d	nt	nt
35-8	0.125	9	27.0 cd	nt	nt	69.1 d	nt	nt	186.7 d	nt	nt	149.7 d	nt	nt
41-5	0	3	97.3 d	100	0	186.7 d	100	0	79.2 d	100	0	92.9 d	100	0
41-5	0	9	41.2 cd	100	0	81.4 d	100	0	54.7 d	100	0	77.1 d	100	0
41-5	0.125	3	13.5 bcd	100	0	82.1 d	100	0	79.9 d	100	0	84.8 d	100	0
41-5	0.125	9	5.9 bc	100	0	23.8 cd	100	0	52.3 d	100	0	70.4 cd	100	0
44-1.5	0	3	11.2 bcd	100	0	27.8 cd	100	100	53.2 d	100	33	60.9 d	100	67
44-1.5	0	9	2.9 abc	100	33	13.5 bcd	100	0	49.8 cd	100	0	50.7 cd	100	0
44-1.5	0.125	3	3.1 abc	100	0	12.0 bcd	100	33	8.1 bc	100	0	13.7 bcd	100	0
44-1.5	0.125	9	2.4 abc	100	33	5.7 bc	100	67	7.1 bc	100	0	6.8 b	100	0
44-5	0	3	1.3 ab	100	67	2.9 abc	67	0	12.8 bcd	100	0	9.9 bc	67	33
44-5	0	9	0.1 ab	100	0	0.5 ab	100	0	6.9 bc	100	0	2.5 ab	100	0
44-5	0.125	3	1.1 ab	100	0	1.4 ab	100	0	1.2 ab	100	0	0.9 a	33	0
44-5	0.125	9	0.3 ab	100	33	0.5 ab	33	0	0.7 a	100	0	0.0 a	33	0
47-3	0	3	0.0 ab	0	0	0.1 ab	0	0	0.2 a	100	0	0.0 a	0	0
47-3	0	9	0.0 ab	0	0	0.0 ab	0	0	0.1 a	100	0	0.0 a	0	0
47-3	0.125	3	0.2 ab	0	0	0.1 ab	0	0	0.1 a	100	0	0.0 a	50	0
47-3	0.125	9	0.0 ab	0	0	0.0 ab	0	0	0.0 a	100	0	0.0 a	100	0

[t] Temperature regimes that simulated solarization consisted of temperatures increased daily to 35°, 41°, 44°, 44°, or 47°C for 8, 5, 1.5, 5 or 3 hours, respectively; the temperature for the remainder of each day was maintained at 25°C.

[u] Days = duration of temperature regime.

[v] PPG = propagules per gram of soil; the soil was initially infested with 500 chlamydospores per gram.

[w] I = infection.

[x] M = mortality.

[y] Main effect means followed by the same letter in each column do not differ according to Tukey's Honestly Significant Difference procedure ($P \leq 0.05$); data were transformed to ln(ppg+1) for analysis and presented as weighted means ([Exp {mean}]-1).

[z] nt = not tested.

Table 3.17. The effect of pasteurization of soil from Site 2 (John Allen Smith Farm, Gadsden County, Florida), temperature regimes, and cabbage amendment on the survival of chlamydospores of *Phytophthora nicotianae*, and on the percentages of infection and mortality of tomato seedlings after 30 days of exposure in the previously treated soils.

			Test 1						Test 2					
Temperature			Pasteurized soil			Nonpasteurized soil			Pasteurized soil			Nonpasteurized soil		
Regime[t]	Cabbage (%)	Time[u] (days)	PPG[v]	I[w] (%)	M[x] (%)	PPG	I (%)	M (%)	PPG	I (%)	M (%)	PPG	I (%)	M (%)
35-8	0	3	16.6 bc[y]	nt[z]	nt	36.2 bc	nt	nt	14.2 cd	nt	nt	107.3 d	nt	nt
35-8	0	9	44.2 c	nt	nt	45.8 c	nt	nt	47.3 d	nt	nt	50.1 d	nt	nt
35-8	0.125	3	54.3 c	nt	nt	8.2 abc	nt	nt	1.7 ab	nt	nt	23.4 bcd	nt	nt
35-8	0.125	9	63.8 c	nt	nt	22.4 bc	nt	nt	115.2 d	nt	nt	69.7 d	nt	nt
41-5	0	3	84.2 c	100	0	95.3 c	100	0	39.7 cd	100	0	37.9 cd	100	0
41-5	0	9	46.3 c	100	33	43.7 c	100	0	31.9 bcd	100	33	35.7 bcd	100	0
41-5	0.125	3	1.4 ab	100	0	10.6 abc	100	0	3.0 bc	100	0	10.5 bcd	100	0
41-5	0.125	9	3.2 ab	100	0	9.3 abc	100	0	2.9 bc	100	0	10.0 bcd	100	0
44-1.5	0	3	23.1 c	100	0	35.1 bc	100	67	10.8 bcd	100	0	12.1 bcd	100	0
44-1.5	0	9	6.9 abc	100	0	9.0 abc	100	0	8.9 bcd	100	0	10.5 bcd	100	0
44-1.5	0.125	3	3.4 ab	100	0	3.7 ab	100	0	2.4 abc	100	33	5.3 bc	100	33
44-1.5	0.125	9	4.0 abc	100	0	1.9 ab	100	33	1.0 a	100	0	6.8 bc	100	0
44-5	0	3	1.6 abc	100	0	0.5 a	100	33	1.0 a	100	0	0.5 a	100	0
44-5	0	9	0.0 a	100	0	0.0 a	67	0	0.0 a	100	0	0.2 a	100	0
44-5	0.125	3	0.0 a	100	0	0.6 ab	100	0	0.0 a	100	0	0.4 a	100	0
44-5	0.125	9	0.0 a	100	0	0.0 a	100	0	0.0 a	100	0	0.2 a	100	0
47-3	0	3	0.0 a	0	0	0.0 a	0	0	0.0 a	100	33	0.0 a	33	0
47-3	0	9	0.0 a	0	0	0.0 a	0	0	0.0 a	100	0	0.0 a	0	0
47-3	0.125	3	0.0 a	0	0	0.0 a	0	0	0.0 a	100	33	0.0 a	0	0
47-3	0.125	9	0.0 a	0	0	0.0 a	0	0	0.0 a	100	33	0.0 a	0	0

[t] Temperature regimes that simulated solarization consisted of temperatures increased daily to 35°, 41°, 44°, 44°, or 47°C for 8, 5, 1.5, 5 or 3 hours, respectively; the temperature for the remainder of each day was maintained at 25°C.

[u] Days = duration of temperature regime.

[v] PPG = propagules per gram of soil; the soil was initially infested with 500 chlamydospores per gram.

[w] I = infection.

[x] M = mortality.

[y] Main effect means followed by the same letter in each column do not differ according to Tukey's Honestly Significant Difference procedure ($P \leq 0.05$); data were transformed to ln(ppg+1) for analysis and presented as weighted means ([Exp {mean}]-1).

[z] nt = not tested.

Table 3.18. The effect of pasteurization of soil from Site 3 (NFREC-Quincy, Gadsden County, Florida), temperature regimes, and cabbage amendment on the survival of chlamydospores of *Phytophthora nicotianae*, and on the percentages of infection and mortality of tomato seedlings after 30 days of exposure in the previously treated soils.

			Test 1								Test 2							
Temperature			Pasteurized soil				Nonpasteurized soil				Pasteurized soil				Nonpasteurized soil			
Regime[t]	Cabbage (%)	Time[u] (days)	PPG[v]		I[w] (%)	M[x] (%)	PPG		I (%)	M (%)	PPG		I (%)	M (%)	PPG		I (%)	M (%)
35-8	0	3	46.80	cd[y]	nt[z]	nt	37.47	cd	nt	nt	1000.24	e	nt	nt	123.84	cd	nt	nt
35-8	0	9	60.19	d	nt	nt	39.81	cd	nt	nt	237.88	d	nt	nt	137.10	cd	nt	nt
35-8	0.125	3	62.50	d	nt	nt	13.61	bcd	nt	nt	306.97	de	nt	nt	192.06	cd	nt	nt
35-8	0.125	9	21.46	cd	nt	nt	0.00	ab	nt	nt	567.50	e	nt	nt	138.49	cd	nt	nt
41-5	0	3	93.07	d	100	0	70.09	d	100	0	120.02	cd	100	0	99.58	cd	100	33
41-5	0	9	34.98	cd	100	0	45.99	cd	100	0	91.20	cd	100	33	73.51	cd	100	0
41-5	0.125	3	26.55	cd	100	0	8.40	cd	100	0	110.83	cd	100	0	32.72	bcd	100	0
41-5	0.125	9	17.89	cd	100	0	36.15	cd	100	0	78.52	cd	100	0	3.08	b	100	33
44-1.5	0	3	25.08	cd	100	33	38.21	cd	100	67	54.26	cd	100	33	41.65	bcd	100	33
44-1.5	0	9	9.25	bcd	100	0	23.80	cd	100	67	52.09	cd	100	33	45.34	bcd	100	33
44-1.5	0.125	3	22.08	cd	100	67	26.77	cd	100	33	29.72	bcd	100	0	1.80	a	100	0
44-1.5	0.125	9	7.55	bcd	100	33	18.43	cd	100	67	21.04	bc	100	33	1.80	a	67	33
44-5	0	3	2.28	abc	100	0	6.46	bcd	100	0	11.58	b	100	33	2.98	b	100	0
44-5	0	9	0.23	ab	100	0	1.39	abc	33	0	2.05	a	100	67	0.15	a	100	0
44-5	0.125	3	0.00	ab	100	0	0.25	ab	33	0	1.33	a	100	33	0.96	a	100	0
44-5	0.125	9	0.00	ab	100	0	0.13	ab	33	0	0.62	a	100	33	0.15	a	100	0
47-3	0	3	0.00	ab	0	0	0.00	ab	0	0	0.28	a	0	0	0.00	a	0	0
47-3	0	9	0.00	ab	0	0	0.00	ab	0	0	0.00	a	0	0	0.00	a	0	0
47-3	0.125	3	0.00	ab	0	0	0.14	ab	0	0	0.08	a	0	0	0.00	a	67	0
47-3	0.125	9	0.00	ab	0	0	0.00	ab	0	0	0.00	a	0	0	0.00	a	33	0

[t] Temperature regimes that simulated solarization consisted of temperatures increased daily to 35°, 41°, 44°, 44°, or 47°C for 8, 5, 1.5, 5 or 3 hours, respectively; the temperature for the remainder of each day was maintained at 25°C.

[u] Days = duration of temperature regime.

[v] PPG = propagules per gram of soil; the soil was initially infested with 500 chlamydospores per gram.

[w] I = infection

[x] M = mortality.

[y] Main effect means followed by the same letter in each column do not differ according to Tukey's Honestly Significant Difference procedure ($P \leq 0.05$); data were transformed to ln(ppg+1) for analysis and presented as weighted means ([Exp {mean}]-1).

[z] nt = not tested.

was observed in the highest temperature regime, even in treatments from which a few propagules had been recovered.

The numbers of propagules recovered from the soil from site 2 were generally lower than the numbers recovered from the other two soils in both tests (Tables 3.16, 3.17, 3.18, 3.19; Appendix E). No differences in survival were observed between sites 1 and 3 in test 1, while survival was higher in soils from site 3 than in soils from site 1 in test 2 ($P \leq 0.05$).

The effect of pasteurization of the soil on the survival of *P. nicotianae* was significant in test 1; however, no differences in survival were observed in test 2 (Table 3.19). Populations were lower in pasteurized soil than in nonpasteurized soil in test 1 ($P \leq 0.05$) (Appendix E).

Lower numbers of propagules were recovered from the soils as temperature regimes increased (Tables 3.16, 3.17, 3.18, 3.19; Appendix E). Temperature regimes of 44°C for 1.5 hours or higher were more effective than 35°C for 8 hours or 41°C for 5 hours at reducing the populations of *P. nicotianae* in test 1 ($P \leq 0.05$) (Appendix E). In test 2, survival under each temperature regime was significantly lower than that at each preceding regime.

The incorporation of cabbage amendment into soil significantly reduced the populations of *P. nicotianae* in the soil (Table 3.19; Appendix E). However, differences in populations between amended and nonamended soil exposed to specific temperature regimes were not evident from the analysis (Tables 3.16, 3.17, 3.18).

The duration of the heat treatment significantly affected survival of *P. nicotianae* in test 1, but not in test 2 (Table 3.19). Significantly fewer propagules were recovered in the selective medium after 9 days of heat treatment than after 3 days in test 1 ($P \leq 0.05$) (Appendix E); in contrast, no differences in survival between the two sampling dates were observed in test 2.

Table 3.19. Analysis of variance of the effects of three soils, pasteurization, temperature regimes that simulate daily solarization periods, cabbage amendment, and time on the survival of chlamydospores of *Phytophthora nicotianae*.

Source of variation		Test 1			Test 2		
	df	MS	F	P	MS	F	P
Soil[v]	2	3.88	19.64	0.0001	36.18	267.87	0.0001
Pasteurization[w]	1	3.33	16.81	0.0001	0.35	2.59	0.1086
Temperature[x]	4	190.82	964.25	0.0001	249.99	1850.83	0.0001
Cabbage[y]	1	36.19	182.88	0.0001	38.69	286.47	0.0001
Time[z]	1	10.74	54.27	0.0001	0.44	3.25	0.0725
Soil x Pasteurization	2	4.09	20.66	0.0001	14.05	104.04	0.0001
Soil x Temperature	8	2.50	12.65	0.0001	5.75	42.58	0.0001
Soil x Cabbage	2	1.71	8.64	0.0001	0.16	1.18	0.3105
Soil x Time	2	0.75	3.77	0.0245	2.73	20.20	0.0001
Temperature x Cabbage	4	6.91	34.94	0.0001	7.58	56.09	0.0001
Temperature x Time	4	1.61	8.16	0.0001	5.36	39.69	0.0001
Cabbage x Time	1	0.34	1.74	0.1889	1.29	9.58	0.0022
Soil x Pasteurization x Temperature x Cabbage x Time	87	1.01	5.12	0.0001	1.19	8.78	0.0001
Residual	240	0.20			0.13		

[v] Soils used were from Site 1 (Naples Tomato Growers-Gargiulo farm number 4 in Decatur County, Georgia), Site 2 (John Allen Smith farm located in Gadsden County, Florida), and Site 3 (North Florida Research and Educational Center in Gadsden County, Florida).

[w] Soils (1-kg lots) were either nonpasteurized or pasteurized in a microwave oven at 700 W for 4 minutes, after moisture had been adjusted to 5% (w/w).

[x] Temperature regimes that simulated solarization consisted of temperatures increased daily to 35°, 41°, 44°, 44°, or 47°C for 8, 5, 1.5, 5 or 3 hours, respectively; the temperature for the remainder of each day was maintained at 25°C.

[y] Soil amended or not amended with dry, ground cabbage at a rate of 0.125% (w/w).

[z] Time = number of days of exposure to heat treatment at a given temperature regime.

A significant interaction between soils and pasteurization was observed in both tests (Table 3.19). In test 1, no differences in survival of *P. nicotianae* were observed between pasteurized and nonpasteurized soils from sites 2 and 3, and survival was higher in nonpasteurized than pasteurized soil from site 1 ($P \leq 0.05$) (Appendix E). In test 2, survival was higher in nonpasteurized than pasteurized soil from sites 1 and 2, and lower in pasteurized soil from site 3.

The interaction of soils with temperature regimes was significant in both tests (Table 3.19). Generally, survival of *P. nicotianae* decreased with increasing temperatures in regimes in each of the three soils tested (Tables 3.16, 3.17, 3.18; Appendix E).

The effect of cabbage amendment on the survival of *P. nicotianae* within each soil was significant in test 1, but not in test 2 ($P \leq 0.05$) (Table 3.19). Within each soil the addition of cabbage amendment lowered the number of propagules recovered from the soils (Appendix E).

The effects of interactions of soils with duration of heat treatment on the survival of populations of *P. nicotianae* were significant (Table 3.19). In test 1, lower survival was found after 9 days of heat treatment than after 3 days in soils from sites 1 and 3; no differences in survival between the two sampling dates were observed in soils from site 2 ($P \leq 0.05$) (Appendix E). Lower population levels were detected after 9 days of heat treatment than after 3 days in soils from site 3 in test 2; no differences in survival were observed between the two sampling dates in soils from either site 1 or from site 2 in test 2.

The effect of cabbage amendment on survival of *P. nicotianae* within each temperature regime was significant (Table 3.19). In test 1, cabbage amendment reduced the survival of *P. nicotianae*, except at 47°C for 3 hours ($P \leq 0.05$) (Appendix E). In test 2, no differences in survival were found at 35°C for 8 hours or 47°C for 3 hours daily; at all other temperature regimes the cabbage amendment reduced the populations of *P. nicotianae*.

The influence of the interaction of temperature regimes and time on survival was significant for both tests (Table 3.19). No propagules were recovered after either sampling time at 47°C for 3 hours daily in both tests ($P \leq 0.05$) (Appendix E). Lower recovery of *P. nicotianae* was found after 9 days than after 3 days of heat treatment at 44°C for 1.5 hours and at 44°C for 5 hours in test 1; no differences in survival between the sampling times were observed at 35°C for 8 hours, 41°C for 5 hours or 47°C for 3 hours. In test 2, significantly fewer spores germinated in the selective medium after 9 days of heat treatment than after 3 days in all temperature regimes, except at 44°C for 1.5 hours.

The interaction of cabbage amendment and time in reducing populations of *P. nicotianae* was significant only in test 2 (Table 3.19). Within each cabbage concentration fewer spores were recovered in test 1 after 9 days than after 3 days ($P \leq 0.05$) (Appendix E). In contrast, no differences were observed when cabbage was added to the soils in test 2.

Discussion

The findings of this study are in general agreement with related work on the thermal inactivation of spores of *Phytophthora* spp. (Barbercheck and Von Broembsen, 1986; Benson, 1978; Bollen, 1985; Juarez-Palacios *et al.*, 1991). Bollen (1985) reported that a soil culture of *P. cryptogea* required 30 minutes at 45°C to be completely inactivated, and a soil culture of *P. capsici* had to be heated to 50°C for 30 minutes before oospores was killed. Benson (1978) found that culture disks of *P. cinnamomi* containing chlamydospores were killed after 90 minutes at 39°C or 4.5 minutes at 44°C; in contrast, Barbercheck and Von Broembsen (1986) noted that a suspension of chlamydospores of *P. cinnamomi* in water was inactivated after 10 minutes at 44°C. The differential heat sensitivity of isolates of the same species was demonstrated by Juarez-Palacios *et al.*

(1991). These authors found that chlamydospores of *P. cinnamomi* or oospores of a low-temperature isolate of *P. megasperma* added to soil did not survive 20 minutes at 45°C; in contrast, a high-temperature isolate of *P. megasperma* survived more than 30 minutes at the same temperature. The discrepancies among the values reported in the literature could be related to the different types of substratum used to produce the spores and to the different media used for the heat treatment in each study. It is expected that spore suspensions in water would be inactivated faster than soil cultures saturated with water, which in turn would die faster than spores added to soil at a lower soil water matric potential. These assumptions are based on the differential transmission of heat throughout the substrata, the formation of air pockets, or simply due to the nature of the spores formed in each substratum, as noted by Myers *et al.* (1983) and Katan (1981).

The use of heat to inactivate spores of microorganisms in diverse media began as early as 1920 (Bigelow and Esty, 1920). Soon after that the advantages of the logarithmic transformation for the analysis of the data also were realized (Bigelow, 1921; Smith, 1923). The logarithmic transformation indicated that a constant proportion of the spore population was killed per unit of time, and also served the purpose of linearizing the data. Simple linear regressions, then, could be used for the analysis of the effect of time on the survival of spores at each temperature employed; or, coefficients for lethal dosages (LD_{50} or LD_{90}) could be interpolated from the data. Pullman *et al.* (1981a) demonstrated that the logarithmic relationship between time and temperature on the survival of four plant pathogens was maintained at temperatures below 50°C. However, Anderson *et al.* (1996) and Cole *et al.* (1993) pointed out that the use of the 'log-linear' model assumes that all spores in a population have equal heat sensitivity and that death of an individual is dependent on it receiving sufficient heat. Deviations from this basic assumption have been observed and a vitalistic model has been proposed by these authors. The concept in this theory is that individuals in a population do not have identical heat resistances and that these differences are permanent. It is possible that a better understanding of the

survival of *P. nicotianae* would be achieved by using the vitalistic model described by Cole *et al.* (1993).

In the present study the relationship of time and temperature on the survival of *P. nicotianae* was evaluated over a series of temperatures and times (35° to 53°C for 5 to 120 minutes, and 35° to 47° for 2 to 480 hours), for which a surface response approach allowed the simultaneous analysis of the effects of both time and temperature on the survival of the pathogen. The models that best described survival of *P. nicotianae* were linear-exponential or exponential.

The analysis of the effect of temperature regimes on the survival of *P. nicotianae* indicated that a single model is not adequate to describe the changes in survival over time as temperature increased. At the base temperature regime (35°C for 8 hours daily), none of the models accurately described survival, which was little affected by the temperature regime. As temperature increased, the change in survival was best explained by other models, such as an exponential model, then a model of square root of either time or survival, then a logarithmic model of time, and finally a model of the reciprocal of time. The changes in spore survival that resulted in these models may have occurred as constant numbers of spores died per unit time at lower temperatures, a constant proportion of the population was killed per unit time at intermediate temperatures, and larger proportions of populations were inactivated early during the exposure to the highest temperature.

The use of tomato seedlings as baits to detect survival of *P. nicotianae* in the treated soil demonstrated that this method can be as sensitive as the soil dilution plating procedure, except when residual populations are present (less than 1 propagule per gram of soil). The reasoning for using the baiting test was that heat treatment at temperatures that did not eliminate the pathogen from the soil could possibly have affected the ability of the surviving population to infect and colonize a susceptible host, or to germinate in the presence of an external stimulus, such as root extracts. However, it is clear from these

tests that if *P. nicotianae* is present in the soil, even in residual levels, infection of a tomato seedling can occur. The baiting technique has the following disadvantages: population of the pathogen are not quantified, extensive amounts of time and space may be required to grow the seedlings and incubate the baited soil, and the soil samples may be cross contaminated due to handling procedures.

Amending infested soils with dried, ground cabbage leaves and exposing it to heat treatment generally reduced survival of *P. nicotianae* in the present study. The effectiveness of cabbage amendment could be observed in two ways: first, at a given temperature, less time was required to achieve the same reduction in population as cabbage concentration increased; second, with increasing concentrations of cabbage amendment, lower temperatures were required to achieve the same reduction in populations of the pathogen. Populations of *P. nicotianae* were eliminated after 24 hours at 41°C or after 12 days at 38° and 35°C in soil amended with 0.5% cabbage; after 4 days at 41°C or after 20 days at 35°C only residual populations of the pathogen were detected. After 12 days at 38°C no propagules were detected in soils amended with 0.125% or 0.25% cabbage. These findings contrast with the results obtained in the field work, where no additional reductions in populations of *P. nicotianae* were achieved with cabbage amendments. The amount of cabbage incorporated into the soil in the field experiments ranged from 0.125% to 0.25%; however, leaf pieces were much larger and during the drying period may have been rained on, which could have started a decay process with subsequent loss of volatiles. Furthermore, incorporation of the amendment into the field soil was done by disking, and it could not be determined if uniform distribution throughout the soil profile was attained.

The use of cabbage amendment without any additional soil heating has been shown to be effective for the control of *Aphanomyces euteiches* (Lewis and Papavizas, 1971), or inconsistent for control of *Fusarium oxysporum* f. sp. *conglutinans* (Ramirez-Villapudua and Munnecke, 1987, 1988). Heating cabbage amended soils induces the

volatilization of certain compounds that are fungicidal, as demonstrated by Gamliel and Stapleton (1993b). These authors determined that heating soil amended with cabbage released volatile compounds, such as methanol, isothiocyanates and aldehydes, which could be directly correlated with the inactivation of the spores of *Pythium ultimum* and sclerotia of *Sclerotium rolfsii*. The effect of cabbage amendment appears to be directly proportional to its concentration in the soil, as observed by Ramirez-Villapudua and Munnecke (1988). These authors found that increasing the concentration of cabbage from 0.25% to 2% reduced the population of *F. oxysporum* f. sp. *conglutinans* to undetectable levels in 12 days instead of the 30 days required at the lower concentration. Other crucifers, such as kale (*Brassica oleracea* var. *viridis*) and mustard (*B. nigra*) were also effective for the control *F. oxysporum* f. sp. *conglutinans*. Another factor that may determine the effectiveness of the crucifer amendment is the concentration of glucosinolates present in the crop (Mayton *et al.*, 1996). These authors found that some cultivars of *Brassica* spp. were more effective than others in the control of *F. sambucinum*. Even though Ramirez-Villapudua and Munnecke (1988) demonstrated that dried cabbage residue is more effective than fresh residue for the control of *F. oxysporum* f. sp. *conglutinans*, other *Brassica* spp. may be more effective as fresh amendment, as demonstrated by Subbarao and Hubbard (1996) with broccoli (*B. oleracea* var. *botrytis*) and *Verticillium dahliae*.

One major benefit of the use of intermittent heat in thermal inactivation studies is the provision of estimations of the effectiveness of soil solarization to control plant pathogens. However, cycling temperatures have not been employed routinely, possibly due to the compounded difficulties of establishing appropriate temperature regimes, the daily requirement of adjusting each temperature, and the longer time required to reach desirable control of the organism under study. Examples of the use of pulsing temperatures are provided by Gamliel and Stapleton (1993b), Porter and Merriman (1983), Tjamos and Fravel (1995) and Wicks (1988).

Wicks (1988) analyzed the effect of intermittent heat on the survival of mycelium of *Phytophthora cambivora*. None of the isolates tested survived after 1 day at a regime of 45°C for 6 hours and 20°C for 18 hours daily. The response of the isolates was variable and inconclusive at either 40° or 35°C for 6 hours daily during 4 days. However, mycelium is not the most likely survival structure of *P. cambivora* in the soil.

Tjamos and Fravel (1995) evaluated intermittent heat on the survival of a suspension of microsclerotia of *Verticillium dahliae* over 4 days. The following daily temperature regimes were used: base temperature of 31°C for 10 hours and high temperature at 35°C for 14 hours, base temperature of 33°C for 10 hours and high temperature at 36°C for 14 hours, and base temperature of 35°C for 10 hours and high temperature at 38°C for 14 hours. After 4 days at the highest temperature regime, less than 1% of the sclerotia germinated. In their study the use of sclerotium suspensions in water negated the insulating effect of air pockets in the soil, and the duration of the high temperature in each regime was longer than that which would normally occur under field conditions.

A more comprehensive study was done by Porter and Merriman (1983) with several soilborne plant pathogens. These authors used infested soil held at field capacity, and over 15 days evaluated cycles of low temperature at 25°C for 18 hours, followed by 6 hours daily at a supplemental temperature of 25°, 30°, 35°, 40°, 45° or 50°C. Survival of each pathogen depended on the heat sensitivity of the type of propagule being evaluated. For example, *V. dahliae* did not survive for 15 days when the high temperature was above 40°C; in contrast, *Pythium irregulare* was recovered at $5x10^4$ propagules per gram of soil at 50°C, even at the end of the experiment.

Gamliel and Stapleton (1993b) selected two temperature regimes similar to those found in the San Joaquin Valley in California to evaluate the effectiveness of the regimes and cabbage amendment on the control of *P. ultimum* and *S. rolfsii*. Both pathogens were eliminated after 4 days in the cabbage amended soil at either temperature regime of 38°C

or 45°C for 4 hours daily plus 20 hours at 30°C; however, propagules of these pathogens could be recovered from the nonamended soil after 4 days at the same temperature regimes.

Comparisons between studies are further complicated due to the differences in length of the experiments, amplitude between low and high temperatures in each regime, and the duration of the high temperature. If a given *in vitro* experiment is to be compared with soil solarization in the field, then the duration of the *in vitro* study should be similar to the solarization trial, and the temperature regimes should simulate those observed during solarization.

In the present study populations of *P. nicotianae* decreased to residual levels after 15 days only when temperature regimes simulating optimum solarization conditions (47°C for 3 hours daily) were used; under these circumstances no infection of tomato seedlings was observed after as little as 3 days of heat treatment. The use of 44°C for 5 hours daily also reduced populations to levels below 1 propagule per gram of soil; however, infection of tomato seedlings exposed to this regime was observed throughout the experiment. The use of temperature regimes simulating average field temperature regimes reduced the populations to at least 40% of the initial infestation level, but all seedlings were colonized at these regimes.

Athough the effect of soil moisture on reproduction and dispersal of *Phytophthora* spp. has been researched extensively (Browne and Mircetich, 1988; Ferrin and Mitchell, 1986; Lutz and Menge, 1991; McIntosh, 1972; Neher and Duniway, 1992; Ristaino *et al.*, 1992; Sidebottom and Shew, 1985a, 1985b), the effect of soil moisture and heat on the inactivation of spores of this genus has not been addressed before. Survival of chlamydospores of *P. nicotianae* was lower in saturated soil (0 kPa) than at the two other soil water matric potentials evaluated (-10 and -30 kPa). The two lower soil water matric potentials (-10 and -30 kPa) used for this study are close to what is normally considered field capacity of a soil and they represent an optimum for thermal inactivation studies.

However, in field studies, these conditions are very difficult to maintain for the time required for soil solarization without supplemental irrigation.

Porter *et al.* (1991) evaluated the effect of two moisture contents (field capacity and 10% of field capacity) and constant temperature on survival of *Plasmodiophora brassicae* in two soils. Only 15 days were required to kill all spores of *P. brassicae* in either soil at 40°C or above when the soil moisture was at field capacity. In dry soil, however, inactivation was observed only at 50° or 55°C.

The effect of soil water matric potential on the thermal inactivation process is twofold. First, temperature maxima of the soil increase with increasing soil moisture content (Mahrer *et al.*, 1984). This principle is important in field experiments, but is not operative in an *in vitro* system as used in the present study. The second effect of moisture is the increase in heat transfer or conduction in the soil, with a subsequent reduction of air pockets that could provide an insulation for the spores (DeVay, 1991a; Stapleton and DeVay, 1986).

One of the key biological principles of soil solarization is that, due to the relatively low temperatures of solar heating, stimulation of microbiological activities in solarized soils may lead to biological control of plant pathogens (Katan, 1985). This indirect effect of soil solarization may be related to a differential thermal sensitivity of other soil microbiota, such as actinomycetes and certain bacteria, or to a greater saprophytic competitive ability of these microorganisms in relation to pathogens once the soil has cooled down (Stapleton and DeVay, 1986). Several researchers have enumerated some of the groups of microorganisms that may be involved in the eventual biological control of plant pathogens in solarized soils (DeVay, 1991; DeVay and Katan, 1991; Gamliel and Katan, 1993; Gamliel and Stapleton, 1993a; Keinath, 1996; Ramirez-Villapudua and Munnecke, 1988; Stapleton, 1991; Stapleton and DeVay, 1984). Gamliel and Stapleton (1993a) determined the microbial activity in the soil using an indirect measure of respiration with fluorescein diacetate. Initially, heated soils had less microbial

activity than nonheated control soil, but after 2 weeks the activity was similar in both soils. Keinath (1996) determined populations of thermotolerant fungi, fluorescent *Pseudomonas* spp., *Bacillus* spp., and actinomycetes by soil dilution plating. Populations of thermotolerant fungi increased during solarization and declined in the months following solarization, but remained higher than in nonsolarized soils. Fluorescent *Pseudomonas* spp. were not found in the soil immediately after solarization; populations of actinomycetes were not affected by solarization; and populations of *Bacillus* spp. increased both in solarized and nonsolarized soils.

All previous work on thermal inactivation of various plant pathogens has been done with spores in water suspension (Benson, 1978; Smith, 1923; Tjamos and Fravel, 1995), with pasteurized soil (Myers *et al.*, 1983), or with naturally infested soil (Bollen, 1985; Juarez-Palacios *et al.*, 1991; Kulkarni *et al.*, 1992; Pullman *et al.*, 1981a). One objective of the present research was to determine if lower survival of *P. nicotianae* occurred in nonpasteurized soils than in pasteurized soils. Two of the soils evaluated (Site 1 and Site 2) were collected from fields where tomato was commercially grown and soil fumigation with methyl bromide was routinely used. In these soils, survival of *P. nicotianae* was either higher in nonpasteurized soils or similar in nonpasteurized and pasteurized soil. In soil from Site 3, which had been weed fallow for several years, survival of *P. nicotianae* was either lower in nonpasteurized soil or similar under both soil treatments. Soils that have been fallow for some time may have a greater diversity of microorganisms that could inhibit, compete with, or lyse the structures of plant pathogens, such as *P. nicotianae*, thus decreasing their survival. In contrast, soils that have been routinely used for commercial production of a crop and have been fumigated with methyl bromide regularly, may provide a biological vacuum after each fumigation, allowing the pathogen to reestablish in the soil and colonize it (Maloy, 1993). Possibly the duration of the tests was too short (9 days) to observe any definite trends in relation to the effects of natural soils and temperature regimes on the inactivation of chlamydospores

of *P. nicotianae*. Although there were limitations in time of exposure in this study, this is the first attempt to determine the biological effects of three nonpasteurized soils on survival of *P. nicotianae* in an *in vitro* system.

CHAPTER 4
SUMMARY AND CONCLUSIONS

The effects of soil solarization and cabbage amendment on the survival of *Phytophthora* spp. were evaluated in North Florida. Soil solarization was applied as a potential soil disinfestation technique in mid-summer of 1994, 1995, and 1996, in five field trials at three sites for 49, 48, 55, 45, and 45 days. During the field trials precipitation occurred on 30, 31, 17, 19, and 21 days, respectively. Ambient air temperature exceeded 35°C only in 1995 and 1996. Soil temperatures exceeded temperature thresholds of 44° and 47°C only under clear, low density polyethylene film and under clear, gas impermeable plastic.

Soil solarization using clear, low density polyethylene film, or with clear, gas impermeable plastic film was an effective strategy for the reduction of populations of *Phytophthora nicotianae* and *P. capsici* in the top 10-cm layer of soil from Site 1 or Site 3. However, at the 25-cm depth, survival of *P. nicotianae* was similar to that in the white-on-black low density polyethylene, and survival of *P. capsici* was similar to that in the nontarped treatment. The incorporation of 70 to 90 metric tons of cabbage into the soil did not affect survival of either pathogen. *Phytophthora nicotianae* could not be quantified in the selective medium for any of the treatments in Site 2 (1994) due to bacterial contamination by *Burkholderia cepacia*, or from the first test in Site 3 (1995). *Phytophthora capsici* was not recovered from any of the trials in Site 3 in 1995 or 1996. In 1996 no oospores of *P. capsici* were observed in the inoculum at the time of soil infestation, and this might have contributed to the lack of survival of this pathogen. Overall conditions for solarization were excellent in 1995; the soil was at field capacity (-

5 kPa), air temperature exceeded 35°C six times, precipitation occurred only on 17 days, and solarization was carried on for 55 days. During the solarization period soil temperatures under the clear, low density polyethylene film exceeded 44° and 47°C for 20 and 6 days respectively. All these factors coupled with the potentially diverse soil microbiota present in a field that had been fallow for several years might account for the lack of survival of the *Phytophthora* spp. in any of the treatments, even the controls.

Some of the possible changes to allow better evaluation of the effectiveness of soil solarization in combination with amendment for the control of *Phytophthora* spp. include: disking the cabbage residue into the soil before making the beds for solarization, and thus promoting a better distribution of the amendment throughout the soil profile; irrigating the field for a few days to ensure that soil moisture is optimum down to 30 cm of depth; and making sure that the appropriate survival structures are present in the inoculum before it is taken to the field.

The temporal analysis of survival of *P. nicotianae* in soil from Site 3 indicated that populations of the pathogen declined with time at the 10-cm depth in plots that were solarized from 17 to 45 days. At the 25-cm depth and in the nontarped treatment, populations of *P. nicotianae* were not affected. No propagules of the pathogen were recovered at either depth or sampling date from soil treated with methyl bromide. Further studies following the course of inactivation of survival structures, such as chlamydospores or oospores, during soil solarization would help to determine the minimum time required for soil solarization to effectively control *Phytophthora* spp. in the soil.

The time required to eliminate *P. nicotianae* from the soil in laboratory thermal inactivation studies decreased with increasing temperatures. Populations of the pathogens were reduced from the infestation level of 500 chlamydospores per gram of soil at the beginning of the tests to 0.2 propagule per gram of soil after 10 minutes at 53°C, 45 minutes at 50°C, 4 hours at 47°C, 12 hours at 44°C, 4 days at 41°C, and 16 days at 38°C.

More than 10% of the initial population survived for 20 days at 35°C . The incorporation of cabbage amendment into the soil reduced the time required to inactivate the chlamydospores of *P. nicotianae* at all temperatures tested. Populations of the pathogen dropped below a detectable level after 4 days in soil amended with 0.5% cabbage and heated to 35°C. Populations also declined below a detectable level in soils amended with 0.125, 0.25, and 0.5% cabbage and heated at 38°C. Similar reductions in populations of the pathogen were not observed in the field experiment, possibly due to the different procedures in the preparation and incorporation of cabbage into the soil. For the laboratory experiments, cabbage wrapper leaves were air dried in a greenhouse, ground in a Wiley mill with a 20-mesh screen; in contrast, for the field test, cabbage heads and wrapper leaves were chopped with a machete, or shredded in a mechanical shredder, air dried for 1 week on the soil in the plots and disked into the soil as the solarization beds were prepared. These differences may have resulted in a loss of potential volatiles during the drying period and uneven incorporation of the amendment throughout the soil profile, which may have prevented the release of volatiles at the lower depths of the soil (25 cm).

Although no propagules were detected by the soil dilution plating in soils maintained at 41°C, the presence of the pathogen was detected by the tomato seedling baiting technique. Detection of *P. nicotianae* by the baiting technique provided similar results to the soil dilution plating procedure, except when residual populations were present in the soil (less than 0.2 propagule per gram of soil). At this low level the pathogen was more frequently detected by one or the other method due to the restricted volume of soil used in the assays.

The use of temperature regimes that simulated solarization periods increased the time required to inactivate the chlamydospores of *P. nicotianae* in relation to the use of constant temperature. Of all temperature regimes evaluated, only those simulating optimum solarization periods (44°C for 5 hours daily and 47°C for 3 hours daily) eliminated the pathogen from the infested, amended soil and prevented mortality of

tomato seedlings; infection was prevented only in soil treated at the highest temperature regime. All other temperature regimes (35°C for 8 hours daily, 41°C for 5 hours daily and 44°C for 1.5 hours daily) did not prevent infection or mortality of the seedlings.

One of the few factors that can be controlled during soil solarization is the soil water matric potential through the use of irrigation. The analysis of the effect of soil water matric potential and temperature regimes on the inactivation of chlamydospores of *P. nicotianae* in cabbage amended soils indicated that survival was lowest in saturated soil; and as temperature increased, survival of the pathogen decreased at all soil water matric potentials evaluated (0, -10 and -30 kPa). The soil water matric potentials evaluated represent optimum levels for the study of thermal inactivation; however, under field conditions lower potentials may be found, as in the third trial in Site 3. Extending the range of soil water matric potentials to -50 kPa, or -100 kPa and the treatment time to 15 to 20 days would allow better comparisons with the field data.

The study with different soils and soil pasteurization, coupled with cabbage amendment and heat simulating solarization periods indicated that soils with potentially greater microbiological activity can be more suppressive to populations of *P. nicotianae*. The duration of the tests (9 days) did not allow the full expression of any of the biological factors that may have been present.

Further studies should be conducted on the effects of temperature regimes, such as 44°C for 1.5 or 5 hours daily, on the inactivation of chlamydospores of *P. nicotianae* over time periods similar to those observed during solarization. Another promising area of study is the possibility of inducing suppressiveness of a soil after heat treatment. Such a study could be carried by heating nonpasteurized soils for 15 days, incubating it for another 15 days (average time between the end of a solarization period and transplanting the field with seedlings), and then adding the inoculum and further incubating it to follow the dynamics of the population of the pathogen.

Another area that needs clarification is the relationship of inoculum density of *P. nicotianae* to disease incidence in tomato. The studies in this dissertation indicated that very low levels of the pathogen could cause infection of 1-month-old tomato seedlings, even after the stress of the heat treatments. It is not known how closely the disease responses described here would relate to root infection of tomato with *P. nicotianae* or *P. capsici* and subsequent disease under field conditions.

APPENDIX A
PRODUCTION OF CHLAMYDOSPORES OF *PHYTOPHTHORA NICOTIANAE*

Introduction

Chlamydospores, the primary inoculum of *Phytophthora nicotianae*, overwinter in the soil or on plant tissue, such as roots, or other organic material. Production of chlamydospores in controlled environments has been limited by the requirements of the fungus for specific conditions that may favor the formation of one type of spore over other forms. As an example, chlamydospore production is favored by low temperatures and saturated soils, and sporangium production is favored by high temperatures and high soil moisture, but not saturation. The most commonly used procedure for producing chlamydospores for quantitative studies was described by Tsao in 1971 (Mitchell and Kannwischer-Mitchell, 1992), and it is still being used today. Ioannou and Grogan (1985) described the use of controlled atmosphere for the formation of chlamydospores of *P. nicotianae*. These authors found that whenever the concentration of O_2 was kept between 10 and 12% and the concentration of CO_2 was raised to 6 to 8%, chlamydospores were the only spores formed in liquid culture. The advantages of the method described by Ioannou and Grogan (1985) were the reduced time for the formation of spores, 2 weeks instead of 4, and the use of a growth chamber at 20°C, which alleviated the need to change temperatures for the selective production of chlamydospores. Studies on the formation of chlamydospores in solid organic media have yet to be reported.

The objectives of this study were to determine the effect of a controlled atmosphere environment on the formation of chlamydospores in a solid organic medium,

wheat seeds, and the effect of the soil on the induction of chlamydospore formation in the seeds.

Materials and Methods

Isolate Pn21 of *Phytophthora nicotianae* was maintained in V8-juice agar cultures for use in all experiments. For the studies in liquid media, four plugs from actively growing cultures were transferred to petri plates containing 20 ml of sterile V8-juice broth. After 2 days of incubation at 25°C, the medium was removed by suction; the mycelial mats were then rinsed with sterile distilled water three times at 5-minute intervals. Fifteen milliliters of water were added to the plate, and each plate was placed in a jar maintained with a controlled atmosphere. Controls consisted of plates prepared in the same manner and left outside of the jar on the same shelf of an incubator in which the experiment was conducted.

The cultures in the solid medium were prepared by autoclaving a flask containing 20 g of wheat seeds and 30 ml of distilled water on 2 consecutive days. Four plugs of actively growing mycelium were transferred to each flask and incubated in the dark at 25°C for 2 weeks. The flasks were shaken vigorously twice a week to ensure uniform colonization of the wheat seeds. Ten grams of colonized wheat seeds were transferred to a petri plate, which was placed in a jar for the controlled atmosphere experiment. Controls consisted of plates prepared in the same manner and left outside of the jar on the same shelf of the incubator in which the experiment was conducted

The controlled atmosphere chamber was prepared with 10% O_2, 6% CO_2, and 84% N_2 at a flow rate of 193 ml/min; the temperature was maintained at 20°C. The plates were incubated for 2 weeks in the growth chamber and then removed for determination of the relative number of chlamydospores formed.

The presence of chlamydospores was determined by observing the liquid cultures directly under the microscope, or by pressing the wheat seeds between a cover glass and a glass slide and examining them under the microscope. The number of spores produced in each treatment was estimated by grinding the cultures in 10 ml of water and counting the number of spores in five microscope fields with the combination of a 10X ocular and 10X objective of a microscope, which yielded a real viewfield of 2.5 mm^2.

A second experiment on the effect of soil on the formation of chlamydospores in the solid medium was maintained in two incubators, one at 25°C and the other at 18°C. One gram of the wheat seed inoculum was mixed in 150 g of soil and placed in a 300-ml plastic container with a lid. Soils from the following three sites where solarization was evaluated were either microwaved or left untreated and tested at the two temperatures: Site 1 at the Naples Tomato Growers-Gargiulo farm number 4 in Decatur County, South Georgia; Site 2 at the John Allen Smith farm located in Gadsden County, North Florida; and Site 3 at the North Florida Research and Educational Center in Gadsden County. After 2 weeks of incubation, the containers were removed from the incubators, the inoculum was retrieved by sieving, and the relative abundance of chlamydospores was determined as previously described.

Comparisons among all treatments in each experiment were performed using the procedure PROC GLM of SAS (SAS Institute, Cary, NC; release 6.11 for personal computers) and the means were separated using Tukey's Honestly Significant Difference procedure.

Results and Discussion

The controlled atmosphere was very effective in the selective production of chlamydospores in the liquid cultures ($P \leq 0.05$); in contrast, the plates in the normal atmosphere contained empty sporangia and few chlamydospores (Table 1). The results confirmed the findings of Ioannou and Grogan (1985). However, very few spores were formed on the wheat seeds, although *P. nicotianae* grew on the plates in both the controlled atmosphere and the normal atmosphere. The average diameter of chlamydospores in the liquid medium was 34.5 μm, with a range of 15 to 50 μm.

In the second experiment, more chlamydospores were formed in raw soil at 25°C than in microwaved soil ($P \leq 0.05$) (Table 2). However, at 18°C more spores were found in the microwaved soil. A comparison of relative abundance of chlamydospores formed in the soils indicated that the lower temperature (18°C) was more effective for the production of this type of spore. No chlamydospores were observed on wheat seeds incubated at 25 or 18°C, or on flooded wheat seeds maintained at 18°C. Furthermore, *P. nicotianae* grew in the flooded culture.

It is possible that a depletion of nutrients is required before chlamydospores by *P. nicotianae* are formed, and that more time would be needed for the depletion of the nutrients in wheat seed cultures. Whenever the inoculum is added to soil, competition for nutrients takes place, and *P. nicotianae* apparently responds to the depletion of nutrients by forming chlamydospores. Lower temperatures may limit the intensity of the overall microbial activity and allow *P. nicotianae* to form more chlamydospores before all of the nutrients are depleted.

Table A.1. Number of chlamydospores produced by *Phytophthora nicotianae* after 2 weeks of exposure to a controlled atmosphere.

Medium	Environment	Spore type	Number of spores[x]	
			Average	Standard Deviation
Liquid	10% O_2, 6% CO_2.	chlamydospores	13.00 a[z]	0.74
Liquid	10% O_2, 6% CO_2.	sporangia	0.04 c	0.04
Liquid	air	chlamydospores	6.00 b	0.51
Liquid	air	sporangia	6.00 b	0.44
wheat seeds	10% O_2, 6% CO_2.	chlamydospores	1.00 c	0.20
wheat seeds	10% O_2, 6% CO_2.	sporangia	0.00 c	0.00
wheat seeds	air	chlamydospores	0.00 c	0.00
wheat seeds	air	sporangia	0.00 c	0.00

[x] Average of 27 optical fields observed with a combination of a 10X ocular and a 10X objective (2.5-mm^2 field of view).

[z] Main effect means followed by the same letter do not differ according to Tukey's Honestly Significant Difference procedure ($P \leq 0.05$).

Table A.2. Number of chlamydospores produced by *Phytophthora nicotianae* in wheat seeds buried in three different soils for 2 weeks.

Soil[w]	Microwaving[x]	Temperature (°C)	Number of spores[y]
Site 1	YES	18	60 a[z]
Site 1	YES	25	10 de
Site 1	NO	18	15 d
Site 1	NO	25	27 c
Site 2	YES	18	38 b
Site 2	YES	25	5 e
Site 2	NO	18	14 d
Site 2	NO	25	2 e
Site 3	YES	18	20 cd
Site 3	YES	25	1 e
Site 3	NO	18	11 de
Site 3	NO	25	1 e

[w] Site 1 (Naples Tomato Growers-Gargiulo farm number 4 in Decatur County, Georgia); Site 2 (John Allen Smith farm located in Gadsden County, Florida); Site 3 (North Florida Research and Educational Center in Gadsden County).

[x] Soil microwaved at 700W for 4 minutes in plastic bags containing 1 kg of soil.

[y] Average of 27 optical fields observed in a 2.5-mm^2 field of view.

[z] Main effect means followed by the same letter do not differ according to Tukey's Honestly Significant Difference procedure ($P \leq 0.05$).

APPENDIX B
DETERMINATION OF SOIL WATER MATRIC POTENTIAL

Water relations are among the most important physical phenomena that affect the biology of soilborne plant pathogens. Duniway's (1979) review, "Water Relations of Water Molds," highlights the importance of water in every aspect of the life cycle of this group of organisms. In summary, sporangia of *Phytophthora* spp. are formed at soil water matric potentials ranging from -2.5 kPa to -60 kPa. While saturated soils (0 kPa) may inhibit the formation of sporangia, zoospore release is enhanced and may continue as the matric potential is maintained above -5 kPa. Similarly, zoospore motility has been detected in soils with matric potentials ranging from 0 to -5 kPa. The effects of soil water potentials on chlamydospores has been less extensively studied; however, it is known that water requirements for chlamydospore formation and germination by some species parallel those for sporangial formation.

Generally, biological phenomena in the soil are more closely related to the soil water matric potential than to moisture content. Furthermore, the use of the soil water matric potential allows direct comparisons among soils.

The soil water matric potentials of disturbed soil samples from the following sites were determined with a pressure plate apparatus equipped with a 3-bar ceramic plate (Soilmoisture Equipment Corp., Santa Barbara, CA): Site 1 was located at the Naples Tomato Growers-Gargiulo farm number 4 in Decatur County, Georgia; Site 2 at the John Allen Smith farm located in Gadsden County, Florida; and Site 3 at the North Florida Research and Educational Center in Quincy, Gadsden County. Water retention curves for soil from each of the three sites are presented.

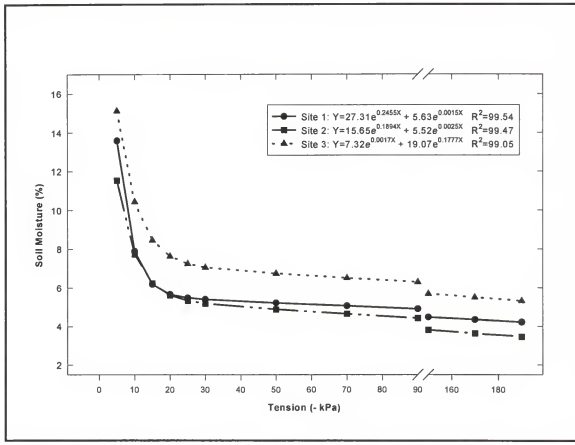

Figure B.1. Moisture retention curves for soils from Site 1 (Naples Tomato Growers-Gargiulo farm number 4 in Decatur County, Georgia), Site 2 (John Allen Smith farm located in Gadsden County, Florida), and Site 3 (North Florida Research and Educational Center in Quincy, Gadsden County).

APPENDIX C
REGRESSION ANALYSES OF THE EFFECTS OF TEMPERATURE REGIMES AND CABBAGE AMENDMENT ON SURVIVAL OF *PHYTOPHTHORA NICOTIANAE*

Regression models generated with Statgraphics Plus were used to characterize the effect of cycling temperatures and cabbage amendment on the inactivation of chlamydospores of *Phytophthora nicotianae*.

Table C.1. Comparison of models used to describe the relationship of exposure time (days) to a temperature regime of 35°C for 8 hours daily in soil amended with 0% cabbage on the survival of *Phytophthora nicotianae* (propagules per gram of soil).

Comparison of Alternative Models

Model	Correlation	R-Squared
Linear	-0.2221	4.93%
Square root-Y	-0.1987	3.95%
Square root-X	-0.1919	3.68%
Exponential	-0.1620	2.62%
Logarithmic-X	-0.1560	2.43%
Multiplicative	-0.0987	0.97%
Reciprocal-X	0.0937	0.88%
S-curve	0.0637	0.41%
Reciprocal-Y	0.0575	0.33%
Double reciprocal	-0.0165	0.03%
Logistic	<no fit>	
Log probit	<no fit>	

Table C.1.1. Regression analysis with the model that best described the relationship of exposure time (days) to a temperature regime of 35°C for 8 hours daily in soil amended with 0% cabbage (T350C) on the survival of *Phytophthora nicotianae* (propagules per gram of soil).

Regression Analysis - Linear model: Y = a + b*X

Dependent variable: T350C
Independent variable: DAYS

Parameter	Estimate	Standard Error	T Statistic	P-Value
Intercept	348.047	20.2675	17.1726	0.0000
Slope	-4.32383	1.66457	-2.59756	0.0105

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	131256.0	1	131256.0	6.75	0.0105
Residual	2.52891E6	130	19453.1		
Total (Corr.)	2.66016E6	131			

Correlation Coefficient = -0.222129
R-squared = 4.93415 percent
Standard Error of Est. = 139.474

Table C.1.2. Model used to compare the relationship of exposure time (days) to a temperature regime of 35°C for 8 hours daily in soil amended with 0% cabbage (T350C) on the survival of *Phytophthora nicotianae* (propagules per gram of soil) with all other temperature regimes (data transformed as ln [ppg+1]).

Regression Analysis - Linear model: Y = a + b*X

Dependent variable: LOG(T350C+1)
Independent variable: DAYS

Parameter	Estimate	Standard Error	T Statistic	P-Value
Intercept	5.71761	0.0780225	73.2815	0.0000
Slope	-0.0120329	0.006408	-1.87779	0.0627

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	1.01654	1	1.01654	3.53	0.0627
Residual	37.4776	130	0.28829		
Total (Corr.)	38.4942	131			

Correlation Coefficient = -0.162504
R-squared = 2.64075 percent
Standard Error of Est. = 0.536926

Table C.2. Comparison of models used to describe the relationship of exposure time (days) to a temperature regime of 35°C for 8 hours daily in soil amended with 0.125% cabbage on the survival of *Phytophthora nicotianae* (propagules per gram of soil).

Comparison of Alternative Models

Model	Correlation	R-Squared
Exponential	-0.5305	28.14%
Reciprocal-Y	0.5286	27.94%
Square root-Y	-0.4753	22.59%
Multiplicative	-0.4367	19.07%
Linear	-0.4137	17.12%
Square root-X	-0.3860	14.90%
Logarithmic-X	-0.3379	11.42%
Double reciprocal	-0.3104	9.64%
S-curve	0.3017	9.10%
Reciprocal-X	0.2200	4.84%
Logistic	<no fit>	
Log probit	<no fit>	

Table C.2.1. Regression analysis with the model that best described the relationship of exposure time (days) to a temperature regime of 35°C for 8 hours daily in soil amended with 0.125% cabbage (T35125C) on the survival of *Phytophthora nicotianae* (propagules per gram of soil).

Regression Analysis - Exponential model: Y = exp(a + b*X)

Dependent variable: T35125C
Independent variable: DAYS

Parameter	Estimate	Standard Error	T Statistic	P-Value
Intercept	4.79999	0.0958888	50.0579	0.0000
Slope	-0.0561875	0.00787536	-7.13459	0.0000

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	22.1647	1	22.1647	50.90	0.0000
Residual	56.6067	130	0.435436		
Total (Corr.)	78.7714	131			

Correlation Coefficient = -0.530453
R-squared = 28.138 percent
Standard Error of Est. = 0.659876

Table C.2.2. Model used to compare the relationship of exposure time (days) to a temperature regime of 35°C for 8 hours daily in soil amended with 0.125% cabbage (T35125C) on the survival of *Phytophthora nicotianae* (propagules per gram of soil) with all other temperature regimes (data transformed as ln [ppg+1]).

Regression Analysis - Linear model: Y = a + b*X

Dependent variable: LOG(T35125C+1)
Independent variable: DAYS

Parameter	Estimate	Standard Error	T Statistic	P-Value
Intercept	4.80793	0.0943055	50.9825	0.0000
Slope	-0.055084	0.00774532	-7.1119	0.0000

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	21.3027	1	21.3027	50.58	0.0000
Residual	54.7528	130	0.421175		
Total (Corr.)	76.0555	131			

Correlation Coefficient = -0.529239
R-squared = 28.0094 percent
Standard Error of Est. = 0.64898

Table C.3. Comparison of models used to describe the relationship of exposure time (days) to a temperature regime of 41°C for 5 hours daily in soil amended with 0% cabbage on the survival of *Phytophthora nicotianae* (propagules per gram of soil).

Comparison of Alternative Models

Model	Correlation	R-Squared
Exponential	-0.8175	66.83%
Reciprocal-Y	0.7844	61.53%
Square root-Y	-0.7778	60.50%
Linear	-0.7141	50.99%
Square root-X	-0.6673	44.53%
Multiplicative	-0.6612	43.72%
Logarithmic-X	-0.5804	33.69%
S-curve	0.4305	18.53%
Double reciprocal	-0.4099	16.80%
Reciprocal-X	0.3504	12.28%
Logistic	<no fit>	
Log probit	<no fit>	

Table C 3.1. Regression analysis with the model that best described the relationship of exposure time (days) to a temperature regime of 41°C for 5 hours daily in soil amended with 0% cabbage (T410C) on the survival of *Phytophthora nicotianae* (propagules per gram of soil).

Regression Analysis - Exponential model: Y = exp(a + b*X)

Dependent variable: T410C
Independent variable: DAYS

Parameter	Estimate	Standard Error	T Statistic	P-Value
Intercept	6.05032	0.092978	65.0725	0.0000
Slope	-0.0912544	0.00685257	-13.3168	0.0000

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	45.6348	1	45.6348	177.34	0.0000
Residual	22.6453	88	0.257333		
Total (Corr.)	68.2801	89			

Correlation Coefficient = -0.817525
R-squared = 66.8347 percent
Standard Error of Est. = 0.50728

Table C.3.2. Model used to compare the relationship of exposure time (days) to a temperature regime of 41°C for 5 hours daily in soil amended with 0% cabbage (T410C) on the survival of *Phytophthora nicotianae* (propagules per gram of soil) with all other temperature regimes (data transformed as ln [ppg+1]).

Regression Analysis - Linear model: Y = a + b*X

Dependent variable: LOG(T410C+1)
Independent variable: DAYS

Parameter	Estimate	Standard Error	T Statistic	P-Value
Intercept	6.04972	0.0921267	65.6674	0.0000
Slope	-0.090339	0.00678983	-13.3051	0.0000

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	44.7238	1	44.7238	177.02	0.0000
Residual	22.2325	88	0.252642		
Total (Corr.)	66.9563	89			

Correlation Coefficient = -0.817285
R-squared = 66.7955 percent
Standard Error of Est. = 0.502635

Table C.4. Comparison of models used to describe the relationship of exposure time (days) to a temperature regime of 41°C for 5 hours daily in soil amended with 0.125% cabbage on the survival of *Phytophthora nicotianae* (propagules per gram of soil).

Comparison of Alternative Models

Model	Correlation	R-Squared
Square root-X	-0.5100	26.01%
Square root-Y	-0.5019	25.20%
Linear	-0.4985	24.85%
Logarithmic-X	-0.4982	24.82%
Exponential	-0.4538	20.60%
Multiplicative	-0.4322	18.68%
Reciprocal-X	0.4025	16.20%
S-curve	0.3359	11.28%
Double reciprocal	-0.1266	1.60%
Reciprocal-Y	<no fit>	
Logistic	<no fit>	
Log probit	<no fit>	

Table C 4.1. Regression analysis with the model that best described the relationship of exposure time (days) to a temperature regime of 41°C for 5 hours daily in soil amended

with 0.125% cabbage (T41125C) on the survival of *Phytophthora nicotianae* (propagules per gram of soil).

Regression Analysis - Square root-X model: Y = a + b*sqrt(X)

Dependent variable: T41125C
Independent variable: DAYS

Parameter	Estimate	Standard Error	T Statistic	P-Value
Intercept	62.46	5.70552	10.9473	0.0000
Slope	-9.52535	1.71251	-5.5622	0.0000

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	13894.0	1	13894.0	30.94	0.0000
Residual	39519.9	88	449.089		
Total (Corr.)	53413.8	89			

Correlation Coefficient = -0.510019
R-squared = 26.0119 percent
Standard Error of Est. = 21.1917

Table C.4.2. Model used to compare the relationship of exposure time (days) to a temperature regime of 41°C for 5 hours daily in soil amended with 0.125% cabbage (T41125C) on the survival of *Phytophthora nicotianae* (propagules per gram of soil) with all other temperature regimes (data transformed as ln [ppg+1]).

Regression Analysis - Linear model: Y = a + b*X

Dependent variable: LOG(T41125C+1)
Independent variable: DAYS

Parameter	Estimate	Standard Error	T Statistic	P-Value
Intercept	3.81191	0.13885	27.4534	0.0000
Slope	-0.0509075	0.0102334	-4.97463	0.0000

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	14.2021	1	14.2021	24.75	0.0000
Residual	50.5024	88	0.573891		
Total (Corr.)	64.7045	89			

Correlation Coefficient = -0.468499
R-squared = 21.9491 percent
Standard Error of Est. = 0.757556

Table C.5. Comparison of models used to describe the relationship of exposure time (days) to a temperature regime of 44°C for 1.5 hours daily in soil amended with 0% cabbage on the survival of *Phytophthora nicotianae* (propagules per gram of soil).

Comparison of Alternative Models

Model	Correlation	R-Squared
Square root-Y	-0.8559	73.25%
Square root-X	-0.8528	72.72%
Exponential	-0.8501	72.27%
Logarithmic-X	-0.8444	71.31%
Linear	-0.8256	68.16%
Multiplicative	-0.8089	65.43%
Reciprocal-Y	0.7170	51.41%
Reciprocal-X	0.7116	50.64%
S-curve	0.6377	40.66%
Double reciprocal	-0.4657	21.69%
Logistic	<no fit>	
Log probit	<no fit>	

Table C 5.1. Regression analysis with the model that best described the relationship of exposure time (days) to a temperature regime of 44°C for 1.5 hours daily in soil amended

with 0% cabbage (T440C) on the survival of *Phytophthora nicotianae* (propagules per gram of soil).

Regression Analysis - Square root-Y model: Y = (a + b*X)^2

Dependent variable: T440C
Independent variable: DAYS

Parameter	Estimate	Standard Error	T Statistic	P-Value
Intercept	9.81598	0.22684	43.2728	0.0000
Slope	-0.259534	0.0167183	-15.5239	0.0000

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	369.127	1	369.127	240.99	0.0000
Residual	134.789	88	1.5317		
Total (Corr.)	503.916	89			

Correlation Coefficient = -0.855872
R-squared = 73.2516 percent
Standard Error of Est. = 1.23762

Table C.5.2. Model used to compare the relationship of exposure time (days) to a temperature regime of 44°C for 1.5 hours daily in soil amended with 0% cabbage (T440C) on the survival of *Phytophthora nicotianae* (propagules per gram of soil) with all other temperature regimes (data transformed as ln [ppg+1]).

Regression Analysis - Linear model: Y = a + b*X

Dependent variable: LOG(T440C+1)
Independent variable: DAYS

Parameter	Estimate	Standard Error	T Statistic	P-Value
Intercept	4.63098	0.0681822	67.9207	0.0000
Slope	-0.0768018	0.00502509	-15.2837	0.0000

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	32.3245	1	32.3245	233.59	0.0000
Residual	12.1775	88	0.138381		
Total (Corr.)	44.502	89			

Correlation Coefficient = -0.852268
R-squared = 72.636 percent
Standard Error of Est. = 0.371996

Table C.6. Comparison of models used to describe the relationship of exposure time (days) to a temperature regime of 44°C for 1.5 hours daily in soil amended with 0.125% cabbage on the survival of *Phytophthora nicotianae* (propagules per gram of soil).

Comparison of Alternative Models

Model	Correlation	R-Squared
Square root-X	-0.6224	38.74%
Logarithmic-X	-0.6151	37.83%
Linear	-0.6003	36.04%
Square root-Y	-0.5796	33.59%
Reciprocal-X	0.5025	25.25%
Exponential	<no fit>	
Reciprocal-Y	<no fit>	
Double reciprocal	<no fit>	
Multiplicative	<no fit>	
S-curve	<no fit>	
Logistic	<no fit>	
Log probit	<no fit>	

Table C 6.1. Regression analysis with the model that best described the relationship of exposure time (days) to a temperature regime of 44°C for 1.5 hours daily in soil amended with 0.125% cabbage (T41125C) on the survival of *Phytophthora nicotianae* (propagules per gram of soil).

Regression Analysis - Square root-X model: Y = a + b*sqrt(X)

Dependent variable: T44125C
Independent variable: DAYS

Parameter	Estimate	Standard Error	T Statistic	P-Value
Intercept	39.6709	2.58388	15.3532	0.0000
Slope	-5.7855	0.775552	-7.45985	0.0000

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	5125.62	1	5125.62	55.65	0.0000
Residual	8105.3	88	92.1057		
Total (Corr.)	13230.9	89			

Correlation Coefficient = -0.622412
R-squared = 38.7397 percent
Standard Error of Est. = 9.59717

Table C.6.2. Model used to compare the relationship of exposure time (days) to a temperature regime of 41°C for 1.5 hours daily in soil amended with 0.125% cabbage (T44125C) on the survival of *Phytophthora nicotianae* (propagules per gram of soil) with all other temperature regimes (data transformed as ln [ppg+1]).

Regression Analysis - Linear model: Y = a + b*X

Dependent variable: LOG(T44125C+1)
Independent variable: DAYS

Parameter	Estimate	Standard Error	T Statistic	P-Value
Intercept	3.45449	0.101241	34.1214	0.0000
Slope	-0.0442587	0.00746156	-5.93156	0.0000

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	10.7346	1	10.7346	35.18	0.0000
Residual	26.8491	88	0.305104		
Total (Corr.)	37.5837	89			

Correlation Coefficient = -0.534433
R-squared = 28.5618 percent
Standard Error of Est. = 0.552362

Table C.7. Comparison of models used to describe the relationship of exposure time (days) to a temperature regime of 44°C for 5 hours daily in soil amended with 0% cabbage on the survival of *Phytophthora nicotianae* (propagules per gram of soil).

Comparison of Alternative Models

Model	Correlation	R-Squared
Logarithmic-X	-0.9536	90.94%
Reciprocal-X	0.9281	86.14%
Square root-X	-0.9133	83.40%
Linear	-0.8525	72.68%
Exponential	<no fit>	
Reciprocal-Y	<no fit>	
Double reciprocal	<no fit>	
Multiplicative	<no fit>	
Square root-Y	<no fit>	
S-curve	<no fit>	
Logistic	<no fit>	
Log probit	<no fit>	

Table C 7.1. Regression analysis with the model that best described the relationship of exposure time (days) to a temperature regime of 44°C for 5 hours daily in soil amended

with 0% cabbage (T440C) on the survival of *Phytophthora nicotianae* (propagules per gram of soil).

Regression Analysis - Logarithmic-X model: Y = a + b*ln(X)

Dependent variable: T440c
Independent variable: DAYS

Parameter	Estimate	Standard Error	T Statistic	P-Value
Intercept	45.5995	1.67579	27.2107	0.0000
Slope	-18.4265	0.919831	-20.0325	0.0000

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	12285.9	1	12285.9	401.30	0.0000
Residual	1224.61	40	30.6153		
Total (Corr.)	13510.5	41			

Correlation Coefficient = -0.953603
R-squared = 90.9359 percent
Standard Error of Est. = 5.5331

Table C.7.2. Model used to compare the relationship of exposure time (days) to a temperature regime of 44°C for 5 hours daily in soil amended with 0% cabbage (T440C) on the survival of *Phytophthora nicotianae* (propagules per gram of soil) with all other temperature regimes (data transformed as ln [ppg+1]).

Regression Analysis - Linear model: Y = a + b*X

Dependent variable: LOG(T440c+1)
Independent variable: DAYS

Parameter	Estimate	Standard Error	T Statistic	P-Value
Intercept	3.88569	0.135962	28.5792	0.0000
Slope	-0.261322	0.0160873	-16.244	0.0000

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	70.0062	1	70.0062	263.87	0.0000
Residual	10.6123	40	0.265308		
Total (Corr.)	80.6185	41			

Correlation Coefficient = -0.93186
R-squared = 86.8364 percent
Standard Error of Est. = 0.515081

Table C.8. Comparison of models used to describe the relationship of exposure time (days) to a temperature regime of 44°C for 5 hours daily in soil amended with 0.125% cabbage on the survival of *Phytophthora nicotianae* (propagules per gram of soil).

Comparison of Alternative Models

Model	Correlation	R-Squared
Reciprocal-X	0.9560	91.39%
Logarithmic-X	-0.8441	71.25%
Square root-X	-0.7517	56.50%
Linear	-0.6611	43.71%
Exponential	<no fit>	
Reciprocal-Y	<no fit>	
Double reciprocal	<no fit>	
Multiplicative	<no fit>	
Square root-Y	<no fit>	
S-curve	<no fit>	
Logistic	<no fit>	
Log probit	<no fit>	

Table C 8.1. Regression analysis with the model that best described the relationship of exposure time (days) to a temperature regime of 44°C for 5 hours daily in soil amended with 0.125% cabbage (T44125C) on the survival of *Phytophthora nicotianae* (propagules per gram of soil).

Regression Analysis - Reciprocal-X model: Y = a + b/X

Dependent variable: T44125C
Independent variable: DAYS

Parameter	Estimate	Standard Error	T Statistic	P-Value
Intercept	-4.77879	0.825204	-5.79104	0.0000
Slope	37.8509	1.83695	20.6053	0.0000

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	5864.72	1	5864.72	424.58	0.0000
Residual	552.52	40	13.813		
Total (Corr.)	6417.24	41			

Correlation Coefficient = 0.955982
R-squared = 91.3901 percent
Standard Error of Est. = 3.71659

Table C.8.2. Model used to compare the relationship of exposure time (days) to a temperature regime of 44°C for 5 hours daily in soil amended with 0.125% cabbage (T44125C) on the survival of *Phytophthora nicotianae* (propagules per gram of soil) with all other temperature regimes (data transformed as ln [ppg+1]).

Regression Analysis - Linear model: Y = a + b*X

Dependent variable: LOG(T44125C+1)
Independent variable: DAYS

Parameter	Estimate	Standard Error	T Statistic	P-Value
Intercept	2.53127	0.254472	9.94716	0.0000
Slope	-0.216177	0.0301095	-7.1797	0.0000

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	47.9075	1	47.9075	51.55	0.0000
Residual	37.1751	40	0.929377		
Total (Corr.)	85.0826	41			

Correlation Coefficient = -0.75038
R-squared = 56.3071 percent
Standard Error of Est. = 0.964042

Table C.9. Comparison of models used to describe the relationship of exposure time (days) to a temperature regime of 47°C for 3 hours daily in soil amended with 0% cabbage on the survival of *Phytophthora nicotianae* (propagules per gram of soil).

Comparison of Alternative Models

Model	Correlation	R-Squared
Reciprocal-X	0.6563	43.07%
Logarithmic-X	-0.5726	32.78%
Square root-X	-0.5082	25.82%
Linear	-0.4466	19.95%
Exponential	<no fit>	
Reciprocal-Y	<no fit>	
Double reciprocal	<no fit>	
Multiplicative	<no fit>	
Square root-Y	<no fit>	
S-curve	<no fit>	
Logistic	<no fit>	
Log probit	<no fit>	

Table C 9.1. Regression analysis with the model that best described the relationship of exposure time (days) to a temperature regime of 47°C for 3 hours daily in soil amended with 0% cabbage (T470C) on the survival of *Phytophthora nicotianae* (propagules per gram of soil).

Regression Analysis - Reciprocal-X model: Y = a + b/X

Dependent variable: T470C
Independent variable: DAYS

Parameter	Estimate	Standard Error	T Statistic	P-Value
Intercept	-0.365705	0.279522	-1.30832	0.1982
Slope	3.42285	0.622231	5.50092	0.0000

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	47.9588	1	47.9588	30.26	0.0000
Residual	63.3954	40	1.58489		
Total (Corr.)	111.354	41			

Correlation Coefficient = 0.656267
R-squared = 43.0687 percent
Standard Error of Est. = 1.25892

Table C.9.2. Model used to compare the relationship of exposure time (days) to a temperature regime of 47°C for 3 hours daily in soil amended with 0% cabbage (T470C) on the survival of *Phytophthora nicotianae* (propagules per gram of soil) with all other temperature regimes (data transformed as ln [ppg+1]).

Regression Analysis - Linear model: Y = a + b*X

Dependent variable: LOG(T470C+1)
Independent variable: DAYS

Parameter	Estimate	Standard Error	T Statistic	P-Value
Intercept	0.718247	0.134545	5.33833	0.0000
Slope	-0.0569253	0.0159196	-3.5758	0.0009

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	3.32196	1	3.32196	12.79	0.0009
Residual	10.3922	40	0.259806		
Total (Corr.)	13.7142	41			

Correlation Coefficient = -0.492167
R-squared = 24.2228 percent
Standard Error of Est. = 0.509712

Table C.10. Comparison of models used to describe the relationship of exposure time (days) to a temperature regime of 47°C for 3 hours daily in soil amended with 0.125% cabbage on the survival of *Phytophthora nicotianae* (propagules per gram of soil).

Comparison of Alternative Models

Model	Correlation	R-Squared
Reciprocal-X	0.7557	57.10%
Logarithmic-X	-0.6016	36.20%
Square root-X	-0.5111	26.12%
Linear	-0.4342	18.86%
Exponential	<no fit>	
Reciprocal-Y	<no fit>	
Double reciprocal	<no fit>	
Multiplicative	<no fit>	
Square root-Y	<no fit>	
S-curve	<no fit>	
Logistic	<no fit>	
Log probit	<no fit>	

Table C 10.1. Regression analysis with the model that best described the relationship of exposure time (days) to a temperature regime of 47°C for 3 hours daily in soil amended

with 0.125% cabbage (T47125C) on the survival of *Phytophthora nicotianae* (propagules per gram of soil).

Regression Analysis - Reciprocal-X model: Y = a + b/X

Dependent variable: T47125C
Independent variable: DAYS

Parameter	Estimate	Standard Error	T Statistic	P-Value
Intercept	-0.364854	0.136029	-2.68218	0.0106
Slope	2.20952	0.302808	7.29677	0.0000

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	19.9844	1	19.9844	53.24	0.0000
Residual	15.0138	40	0.375344		
Total (Corr.)	34.9982	41			

Correlation Coefficient = 0.755654
R-squared = 57.1013 percent
Standard Error of Est. = 0.612654

Table C.10.2. Model used to compare the relationship of exposure time (days) to a temperature regime of 47°C for 3 hours daily in soil amended with 0.125% cabbage (T47125C) on the survival of *Phytophthora nicotianae* (propagules per gram of soil) with all other temperature regimes (data transformed as ln [ppg+1]).

Regression Analysis - Linear model: Y = a + b*X

Dependent variable: LOG(T47125C+1)
Independent variable: DAYS

Parameter	Estimate	Standard Error	T Statistic	P-Value
Intercept	0.455171	0.099209	4.588	0.0000
Slope	-0.0402598	0.0117386	-3.4297	0.0014

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	1.6616	1	1.6616	11.76	0.0014
Residual	5.65034	40	0.141259		
Total (Corr.)	7.31195	41			

Correlation Coefficient = -0.476702
R-squared = 22.7245 percent
Standard Error of Est. = 0.375844

APPENDIX D

SUMMARY TABLE OF THE STATISTICAL ANALYSIS OF THE EFFECT OF SOIL WATER MATRIC POTENTIAL, TEMPERATURE REGIMES, AND CABBAGE AMENDMENTS ON THE THERMAL INACTIVATION OF CHLAMYDOSPORES OF *PHYTOPHTHORA NICOTIANAE*

Table D.1. Summary statistics for the effect of soil water matric potential, temperature regimes, and cabbage amendments on the survival of *Phytophthora nicotianae*

Factor	Propagules per gram of soil	
	Test 1	Test 2
Matric Potential (-kPa)		
0	26.8 [z] a	21.3 a
10	31.4 b	28.3 c
30	32.9 b	25.0 b
Temperature Regime		
35-8	234.2 e	163.9 e
41-5	155.5 d	84.1 d
44-1.5	34.8 c	21.3 c
44-5	6.7 b	10.3 b
47-3	0.6 a	0.7 a
Cabbage Amendment		
Non-amended	32.0 b	25.0 a
Amended (0.125%)	29.0 a	24.4 a
Time (days)		
1 day	37.8 c	33.5 c
2 days	13.0 a	15.3 a
3 days	22.6 b	17.1 b
6 days	79.6 d	43.6 d

(continued)

[z] Main effect means followed by the same letter in each column do not differ according to the Tukey's Honestly Significant Difference procedure ($P \leq 0.05$); data were transformed to ln(ppg+1) for analysis and presented as weighted means ([Exp {mean}]-1).

Table D.1. - continued

Factor	Propagules per gram of soil	
	Test 1	Test 2
Matric Potential x Temperature Regime		
0 x 35-8	280.4 e	197.2 e
0 x 41-5	99.2 d	50.9 d
0 x 44-1.5	28.9 c	18.5 c
0 x 44-5	6.8 b	9.8 b
0 x 47-3	0.2 a	0.2 a
10 x 35-8	202.3 e	161.0 e
10 x 41-5	201.3 d	104.7 d
10 x 44-1.5	36.8 c	24.3 c
10 x 44-5	6.2 b	13.1 b
10 x 47-3	0.7 a	1.0 a
30 x 35-8	226.4 e	138.7 e
30 x 41-5	188.4 d	111.6 d
30 x 44-1.5	39.6 c	21.5 c
30 x 44-5	7.0 b	8.3 b
30 x 47-3	0.9 a	1.0 a
Matric Potential x Amendment		
0 x 0%	32.6 b	29.4 b
0 x 0.125%	22.0 a	15.4 a
10 x 0%	32.6 a	25.0 a
10 x 0.125%	30.4 a	32.1 b
30 x 0%	31.0 a	21.3 a
30 x 0.125%	36.3 b	29.2 b
Matric Potential x Time		
0 x 1	27.7 c	26.6 c
0 x 2	12.3 a	14.0 a
0 x 3	22.2 b	14.8 b
0 x 6	73.3 d	40.2 d
10 x 1	43.3 c	42.4 c
10 x 2	12.8 a	17.1 a
10 x 3	21.7 b	18.6 b
10 x 6	81.0 d	47.4 d
30 x 1	45.0 c	33.3 c
30 x 2	13.8 a	14.9 a
30 x 3	23.9 b	18.1 b
30 x 6	85.0 d	43.5 d

(continued)

Table D.1. - continued

Factor	Propagules per gram of soil	
	Test 1	Test 2
Temperature Regime x Amendment		
35-8 x 0%	251.9 b	205.0 b
35-8 x 0.125%	217.7 a	131.1 a
41-5 x 0%	222.4 b	130.2 b
41-5 x 0.125%	108.8 a	54.3 a
44-1.5 x 0%	23.8 a	11.8 a
44-1.5 x 0.125%	50.7 b	38.0 b
44-5 x 0%	8.9 b	10.8 a
44-5 x 0.125%	4.9 a	9.7 a
47-3 x 0%	0.4 a	0.5 a
47-3 x 0.125%	0.7 b	1.0 b
Temperature Regime x Time		
35-8 x 1	250.2 b	158.3 b
35-8 x 2	280.9 b	306.5 c
35-8 x 3	216.3 a	154.3 b
35-8 x 6	197.7 a	96.3 a
41-5 x 1	212.3 c	147.9 b
41-5 x 3	154.4 b	71.1 a
41-5 x 6	111.1 a	56.5 a
44-1.5 x 1	56.0 c	38.6 b
44-1.5 x 3	33.2 b	16.7 a
44-1.5 x 6	22.5 a	14.8 a
44-5 x 1	10.6 c	15.0 c
44-5 x 2	6.7 b	10.0 b
44-5 x 3	4.0 a	7.1 a
47-3 x 1	1.5 b	2.3 b
47-3 x 2	0.3 a	0.3 a
47-3 x 3	0.2 a	0.2 a
Amendment x Time		
0% x 1	37.5 c	33.3 c
0% x 2	13.6 a	15.4 a
0% x 3	23.4 b	17.5 b
0% x 6	94.6 d	45.1 d
0.125% x 1	38.1 c	33.7 c
0.125% x 2	12.4 a	15.1 a
0.125% x 3	21.8 b	16.7 b
0.125% x 6	67.0 d	42.1 d

APPENDIX E

SUMMARY TABLE OF THE STATISTICAL ANALYSIS OF THE EFFECT OF THREE DIFFERENT SOILS, SOIL PASTEURIZATION, TEMPERATURE REGIMES, AND CABBAGE AMENDMENT ON THE THERMAL INACTIVATION OF CHLAMYDOSPORES OF *PHYTOPHTHORA NICOTIANAE*

Table E.1.Summary statistics for the effect of three different soils, soil pasteurization, temperature regimes, and cabbage amendment on the survival of *Phytophthora nicotianae*.

Factor	Propagules per gram of soil	
	Test 1	Test 2
Soil[w]		
Site 1	6.9[x] b	12.0b
Site 2	4.8 a	4.5a
Site 3	6.9 b	14.1c
Pasteurization[y]		
Pasteurized soil	5.5 a	9.6a
Non-pasteurized soil	6.8 b	8.9a
Temperature regime[z]		
35-8	31.2 d	80.2e
41-5	29.9 d	39.3d
44-1.5	10.2 c	13.9c
44-5	0.6 b	1.3b
47-3	0.0 a	0a
Cabbage amendment		
Non-Amended	8.8 b	13.2b
Amended (0.125%)	4.2 a	6.4a
Time (days)		
3 days	7.5 b	9.6a
9 days	5.0 a	8.9a

(continued)

Table E.1. - continued

Factor	Propagules per gram of soil	
	Test 1	Test 2
Soil x Pasteurization		
Site 1 Pasteurized soil	4.8 a	10.6 a
Site 1 Non-pasteurized soil	9.8 b	13.6 b
Site 2 Pasteurized soil	4.7 a	3.4 a
Site 2 Non-pasteurized soil	4.9 a	5.8 b
Site 3 Pasteurized soil	7.2 a	21.9 b
Site 3 Non-pasteurized soil	6.6 a	8.9 a
Soil x Temperature regime		
Site 1 x 35-8	43.4 d	58.5 d
Site 1 x 41-5	42.4 d	72.7 c
Site 1 x 44-1.5	7.4 b	21.9 b
Site 1 x 44-5	0.8 a	2.6 a
Site 1 x 47-3	0.1 a	0.0 a
Site 2 x 35-8	30.9 d	33.9 d
Site 2 x 41-5	18.6 c	14.7 d
Site 2 x 44-1.5	7.2 b	6.0 c
Site 2 x 44-5	0.3 a	0.3 b
Site 2 x 47-3	0.0 a	0.0 a
Site 3 x 35-8	22.6 c	256.7 e
Site 3 x 41-5	33.6 c	55.4 d
Site 3 x 44-1.5	19.2 c	19.5 c
Site 3 x 44-5	0.8 b	1.5 b
Site 3 x 47-3	0.0 a	0.0 a
Soil x Amendment		
Site 1 x 0%	8.5 b	16.4 b
Site 1 x 0.125%	5.6 a	8.8 a
Site 2 x 0%	7.7 b	6.7 b
Site 2 x 0.125%	2.8 a	2.9 a
Site 3 x 0%	10.2 b	20.5 b
Site 3 x 0.125%	4.5 a	9.5 a
Soil x Time		
Site1 x 3 days	8.8 b	11.8 b
Site1 x 9 days	5.4 a	12.3 b
Site2 x 3 days	5.3 a	4.0 a
Site2 x 9 days	4.3 a	4.9 a
Site3 x 3 days	8.8 b	17.5 b
Site3 x 9 days	5.3 a	11.3 a

(continued)

Table E.1. - continued

Factor	Propagules per gram of soil	
	Test 1	Test 2
Temperature regime x Amendment		
35-8 x 0%	37.8 b	79.0 a
35-8 x 0.125%	25.7 a	81.4 a
41-5 x 0%	68.5 b	63.6 b
41-5 x 0.125%	12.7 a	24.1 a
44-1.5 x 0%	15.3 b	30.3 b
44-1.5 x 0.125%	6.6 a	6.0 a
44-5 x 0%	1.0 b	2.4 b
44-5 x 0.125%	0.3 a	0.5 a
47-3 x 0%	0.0 a	0.0 a
47-3 x 0.125%	0.0 a	0.0 a
Temperature regime x Time		
35-8 x 3 days	31.5 a	52.7 a
35-8 x 9 days	30.9 a	121.8 b
41-5 x 3 days	37.0 b	47.7 b
41-5 x 9 days	24.1 a	32.4 a
44-1.5 x 3 days	14.7 b	15.1 a
44-1.5 x 9 days	6.9 a	12.7 a
44-5 x 3 days	1.1 b	2.0 b
44-5 x 9 days	0.2 a	0.7 a
47-3 x 3 days	0.1 a	0.0 a
47-3 x 9 days	0.0 a	0.0 a
Cabbage Amendment x Time		
0% x 3 days	11.0 b	14.6 b
0% x 9 days	7.0 a	11.9 a
0.125% x 3 days	5.0 b	6.2 a
0.125% x 9 days	3.5 a	6.6 a

[w] Site 1 = Naples Tomato Growers-Gargiulo farm number 4 in Decatur County, Georgia; Site 2 = John Allen Smith farm located in Gadsden County, Florida; Site 3 = North Florida Research and Educational Center in Gadsden County.

[x] Main effect means followed by the same letter in each column do not differ according to the Tukey's Honestly Significant Difference procedure ($P \leq 0.05$); data were transformed to ln(ppg+1) for analysis and presented as weighted means ([Exp {mean}]-1).

[y] Soil (1-kg lots) was pasteurized in a microwave oven at 700 W for 4 minutes, after moisture had been adjusted to 5% (w/w).

[z] Temperature regimes that simulated solarization consisted of temperatures increased daily to 35°, 41°, 44°, 44°, or 47°C for 8, 5, 1.5, 5 or 3 hours, respectively; the temperature for the remainder of each day was maintained at 25°C.

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BIOGRAPHICAL SKETCH

Lísias Coelho was born on July 3, 1959, in Piumhy, State of Minas Gerais, Brazil. Lísias attended the Universidade Federal de Viçosa in Viçosa, MG, Brazil, between 1979 and 1983, where he earned a Bachelor of Science degree of Engenheiro Florestal. He began a master's program at the same institution immediately after the conclusion of the bachelor's degree. In 1985, he assumed the position of researcher in plant protection at CENIBRA Florestal S.A., in Ipatinga, MG, Brazil. In 1989, Lísias completed his Master of Science degree in plant pathology with a thesis titled "Physiological Variability of *Puccinia psidii* Winter - The Eucalyptus Rust" and married Eliamar Cavaleiro de Moraes Coelho. In the Fall of 1990 Lísias was accepted at the University of Florida to pursue a doctoral degree in the Plant Pathology Department.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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